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## TWO NEW GREGARINIDA.<sup>1</sup>

By JAMES F. PORTER.

### I. *A Gregarine from Clymenella.*

(*Monocystis clymenellae* n. sp.)

THERE is found in the body cavity of *Clymenella torquata* a gregarine which I have been unable to identify with any described species. It resembles, however, in many ways *Monocystis magna* from the earthworm, recently described by Wolters,<sup>2</sup> a species to which it is probably closely allied, though parasitic in a salt-water annelid.

On cutting open a parasitized *Clymenella* one finds in the body cavity, but generally in the posterior half only, numerous white, opaque, oval bodies a little larger than starfish eggs. Microscopic examination shows that these are gregarines in the encysted condition.

The cysts are often found scattered singly through the body wall; but in much-infested worms they are more frequently met with in clusters of as many as eight, ten, or even more, and are surrounded by loose connective tissue.

<sup>1</sup> Contributions from the Zoölogical Laboratory of the Museum of Comparative Zoölogy at Harvard College, under the direction of E. L. Mark, No. LXXXII.

<sup>2</sup> Arch. für mikr. Anatomie, Bd. XXXVII, 1890, pp. 99-134.

A fair idea of the number and relative position of the cysts in the body cavity of the host can be obtained from Pl. I, Fig. 1, which was drawn from a cross section of a greatly infested worm, and shows sections of eighteen gregarine cysts.

Ordinarily the cysts appear white and opaque (Pl. I, Fig. 2); mature specimens, however, when illuminated from beneath, are quite translucent and are seen to be filled with sporocysts.

I have not been able to throw much light on the question, whether or not there is a conjugation or a division preceding the beginning of spore formation. However, the few cases observed which have any bearing on this question seem to point to a conjugation. In the only case of the unencysted condition found (Pl. I, Fig. 3), a division or conjugation—unfortunately one cannot say positively which—is in the act of taking place. This case alone cannot be taken as satisfactory evidence of either process; but in a dozen or more recently encysted gregarines I have noticed a constriction of the protoplasm which strongly suggests a conjugation, the evidence of which has not yet been fully obliterated (Pl. I, Fig. 4). I infer this because, apparently, in this form no division of the protoplasm precedes spore formation (Pl. I, Figs. 5, 9, 10, 12). It therefore seems probable that the case represented in Pl. I, Fig. 3, is a conjugation preparatory to encystment. The close proximity of the two nuclei, which might at first sight be taken as evidence of recent division, is a condition which is entirely in harmony with the ordinary method of conjugation in monocystic gregarines, where, as is well known, the corresponding (anterior) ends of the copulating individuals are the ones to come in contact and fuse.

It is to be inferred that the time intervening between encystment and the formation of the first sporogonia is very short, because the number of cysts found either filled with sporocysts or in the process of spore formation is very large, the number of those in other conditions being comparatively small.

Before the development of spores actually begins, however, the nucleus breaks up (Pl. I, Fig. 6) and eventually disappears entirely (Pl. I, Fig. 7). During this process the character of the protoplasm changes from a loose vacuolated state to a more



compact and finely granular condition. Thus at  $pr'pl''$  (Pl. I, Fig. 6) the protoplasm is still extensively vacuolated, while at  $pr'pl'$  this, more properly speaking, frothy condition has given place to fine granules, some of which are shown highly magnified in Pl. I, Fig. 8. The compact condition of the protoplasm at the close of this stage is shown by the numerous cracks running through hardened specimens (Pl. I, Fig. 7). The cracks are due, of course, to the method of hardening.

I believe that the disintegration of the nucleus continues until the chromatic matter no longer exists as such; at any rate, until it becomes divided into particles so fine as to be invisible even under the highest powers of the microscope. One of the reasons for believing that a complete dissolution of the nucleus takes place is that with its disappearance the chemical character of the protoplasm seems to change. I believe that the protoplasm then develops slightly acid qualities, for I have found that it acts on the haematoxylin stain, turning it gradually to a reddish tint, while the tissue enveloping the cyst retains its original blue color. This is very noticeable in my old slides, where all the cysts in this stage of development stand out as red spots from their blue surroundings (Pl. I, Fig. 7).

Very frequently the first sporogonia are formed before the network state of the protoplasm has been completely replaced by the finely granular condition. The protoplasm shrinks away from the wall of the cyst and then sends out homogeneous, translucent, amoeboid processes from the whole or part of its periphery (Pl. I, Figs. 9, 10). The granules of the central mass are carried out through these processes and, collecting together in clusters, help to form the sporogonia (Pl. I, Fig. 11).

Meanwhile numerous chromatic particles appear, which, by arranging themselves into rings (Pl. I, Figs. 11, 14, and Pl. II, Fig. 18), form the nuclei of the sporogonia.

As more and more sporogonia are formed, the central mass of protoplasm becomes smaller and smaller, large vacuoles appear, and sporogonia are likewise seen in these (Pl. I, Fig. 12). A fine filamentous network can be distinguished in the cyst (*fil, rtl*, Pl. I, Fig. 12); it is perhaps a useless remnant of the original protoplasmic mass.

The process of spore formation seems to be somewhat different in the later stages of sporulation; for, as the protoplasm becomes riper, sporogonia can be seen developing not only at its periphery but also within it (Pl. I, Fig. 13, *spo'go'*). During this process the particles of chromatin appear at first to collect together in large masses (Pl. I, Fig. 13, *chr*) and then to be distributed to the forming sporogonia. At length the protoplasm that still remains breaks up into small masses (Pl. I, Fig. 15, *pr'pl*) and finally becomes employed in the formation of additional sporogonia. One frequently finds, however, in mature cysts—that is, those containing only ripe sporocysts—a small mass of loose protoplasm left over when the formation of the sporogonia has ceased.

The sporogonia that are formed first do not wait in their development for those that are formed later, but continue their growth and metamorphosis without interruption, so that one finds in the same cyst sporogonia in all stages, from such as are just formed to those that are mature (Pl. I, Fig. 15, *spo'go* and *spo'go'*, and Pl. II, Fig. 16).

In one case, I have noticed within the cyst at about this stage delicate transparent membranes (Pl. I, Fig. 15, *mb*). What their purpose is I cannot say, unless possibly they are connected with the formation of the sporocysts.

The development of the sporogonia into sporocysts each containing eight spores, is as follows: The nucleus first, apparently without mitotic changes, divides into two (Pl. II, Fig. 20), then each of these divides, making four (Pl. II, Fig. 21), and finally each of the four divides, thus producing in all eight nuclei (Pl. I, Figs. 15, *spo'go*, and Pl. II, Fig. 17). The example shown in Pl. II, Fig. 19, seems to be an exception to this rule, for here there is a well-developed nucleus at one pole, and near the other a collection of chromatic particles not very compactly arranged and without nuclear membrane or precise boundary. The only explanation I can offer is that abnormally the division has resulted in nuclei of very unequal size.

After their formation the sporogonia increase greatly in size. Pl. II, Fig. 17, shows five sporogonia, four before any division of the nuclear substance, and one after the three successive



divisions which result in eight nuclei. After the first nuclear division the resulting nuclei migrate to opposite poles of the sporogonium (Pl. II, Fig. 20  $\beta$ ). When the second division has occurred, two of the nuclei occupy the two poles of the sporogonium; the remaining two lie on opposite sides of the chief axis, half-way between the poles (Pl. II, Fig. 21). I am unable to say what the positions of the eight nuclei are at the close of the final division. The chromatic substance frequently has the form of eight rings (Pl. II, Fig. 17), and these are usually arranged near the periphery of the cyst. With the metamorphosis of the rings into more homogeneous and elongated nuclei, they take up positions in the periphery of the cyst at or near its equator. It is frequently seen in cross sections (Pl. II, Fig. 24) that the nuclei lie very near the periphery, and closely approximated in two rows of four each, which occupy opposite sides of the cyst. It is possible that each of the clusters is descended from one of the two nuclei resulting from the first division of the nucleus of the sporogonium; but I have not satisfactory proof of this. In other cases (Pl. II, Fig. 25) the nuclei lie near the periphery, but are not thus evenly divided in their grouping. In still other cases there is no evidence of a particular grouping; but this condition may be due to disarrangement caused by mechanical influences in killing, hardening, etc.

During the metamorphosis of the nuclear substance the sporogonia produce two enveloping membranes: an outer one, which I shall call the capsule, and an inner one, which I shall call the cyst (Pl. II, Figs. 22, 23).

The outer envelope is a very transparent, unstainable structure of an elongated cylindrical or spindle-shaped form. It apparently has two openings, one at either end (Pl. II, Fig. 31). Around each opening the wall of the capsule is thickened (*a*, Pl. II, Figs. 22, 23, 29, 31). This thickening constitutes a somewhat rigid ring around the opening of the capsule, which is thus perhaps prevented from being accidentally closed. The two ends of the capsule generally differ slightly from each other; usually one end has more of a neck-like prolongation than the other, and it is this end which generally presents the

more prominent ring. In some cases, however (Pl. II, Fig. 31), the ring at the other end is the thickest. It is not always possible to make out an opening at the end opposite that which is prolonged into a neck (Pl. II, Fig. 23); but when it does exist it may be larger than the opening at the neck end (Pl. II, Figs. 29, 31). A certain amount of shriveling is sometimes to be observed on the part of the capsule, but this is less likely to affect the neck end than the opposite end (Pl. II, Figs. 22, 29, 30). The orifice at the neck end of the capsule varies in diameter from about that of a spore to two or three times that size.

The inner envelope, which does not fill the capsule completely, although of a more or less spindle-shaped form, is much shorter than the capsule, but of nearly the same diameter, so that the space left between the capsule and the cyst is principally at the ends. The cyst is really flask-shaped, having but a single orifice, which terminates in a more (Pl. II, Fig. 22) or less (Pl. II, Figs. 23, 30) neck-like prolongation of the end corresponding to that end of the capsule which I have called the neck end. The opposite end of the cyst is rounded or slightly conical. The orifice sometimes exhibits a thickened rim; it varies a little in diameter, being usually somewhat smaller than that of the capsule, and of a size sufficient to allow the passage of only one spore at a time (Pl. II, Figs. 22, 29, 32).

In the early stages of spore formation the cyst is completely filled by its protoplasmic contents. In later stages nearly or quite all of the contents is concentrated into the spores, which then by no means fill the cyst completely.

Very frequently the whole cyst and contents slip out of the capsule (Pl. II, Figs. 26, 32). Not knowing this, I was at first greatly perplexed at being unable, as I supposed, to stain the spores. I prepared my slides by first breaking open the living cysts by slight pressure on the cover glass, and then killing, fixing, and staining on the slide, as one would in preparing bacteria. After such treatment I almost always found, much to my astonishment, that while thousands of sporocysts remained fixed to the slide, nothing was stained. I attributed this to the inadequacy of the stain, and not until after trying



many experiments with a dozen or more different stains did I discover that there was nothing left to stain, that the cysts containing the spores had escaped from the capsules and had been washed away.

I have not been able to determine how either of the envelopes is formed, but they are both undoubtedly secreted by the protoplasm of the sporogonium, the capsule first, the cyst afterwards.

The protoplasm in the cyst gradually collects into cord- or band-like structures apparently in connection with the elongated nuclei, of which they appear as though tail-like appendages (Pl. II, Figs. 22, 23). The bands at first are only feebly stainable; later they stain more deeply. Large globules of a colorless substance appear in the cyst at the same time; finally, the protoplasm becomes entirely consumed in the formation of the tails.

At the time of the first appearance of the tail-like protoplasmic portions of the spores, the nuclei are elongated, cylindrical rods, usually constricted slightly in several (3 to 5) places, so as to present a rather evenly monilate form. With the completion of the protoplasmic portion of the spore, the chromatic mass becomes shorter and somewhat thicker, and stains more deeply than formerly. It finally assumes an appearance as though made up of only two oval bodies of equal size united end to end. Each portion is about twice as long as thick, and the union of the two is often so intricate that there is only a trace or even no evidence of a constriction, and the nucleus then is a single oval body some three or four times as long as thick.

The tail portion of the immature spores is apt to contain vacuoles, and to shrink somewhat in the process of hardening (Pl. II, Fig. 27). The spores represented in Pl. II, Fig. 28, are abnormal, or else have been subjected to too great pressure.

In many of the sporocysts I have found the spores arranged in a regular manner, with their head ends, that is, their nucleated ends, in or near the equatorial plane, four of their tails being turned in one direction and four in the opposite. In fully as many cases, however, the spores had apparently no definite arrangement in the cyst (Pl. II, Fig. 32).

The fate of the spores after their escape is still unknown. They are so minute that it would be almost impossible to identify them in the tissues of the host. I have found, however, imbedded in the wall of the intestine of *Clymenella*, on the body-cavity side, numerous large amoeboid cells, evidently foreign organisms (Pl. II, Figs. 34-36); these, I think, may possibly be the early amoeba-like stage of this gregarine developed directly from spores, but I have seen no intermediate stages.

After trying many methods of killing and many kinds of stains, I found that the best results were invariably obtained by killing in a concentrated aqueous solution of corrosive sublimate, and staining in Kleinenberg's haematoxylin. The aqueous stains were, as a rule, unsatisfactory, although I succeeded very well with Mayer's aqueous haemalum.

Notwithstanding the greatest care in hardening the specimens and in passing them gradually into chloroform or into oil of thyme, I could not prevent the sporocysts from shrinking more or less. Pl. II, Fig. 33, represents a portion of a section through a nearly mature gregarine cyst; it shows sections through sporocysts in various directions and the great amount of shrinkage that has taken place.

The best way that I have found for preparing slides of sporocysts consists in staining the gregarines *in toto* and breaking open the cysts with needles after they have been transferred to balsam ready for mounting.

About one-fourth of all the worms I have examined were infested. I have collected *Clymenella* as late as the last of November and as early as the middle of April, and have never failed to find infested worms.

My material was collected at Woods Holl, and at the mouth of the Saugus River, near Lynn, Massachusetts.

## II. *A Gregarine from Rhyncobolus.*

While collecting *Clymenella* I frequently dug up fine specimens of *Rhyncobolus Americanus*, Verrill. Curiosity led me to search the intestines of a few of these, where, to my sur-



prise, I discovered a very large gregarine belonging to the order Polycystidea. After this I naturally examined every specimen of *Rhyncobolus* that came in my way; but I found that the parasitized worms were rare,—perhaps 10% of those examined,—and even these contained only a few gregarines, seven or eight at most.

This gregarine is generally found with its anterior, that is, the larger, end (Pl. III, Fig. 37) buried in the wall of the host's intestine, and its posterior end projecting out into the intestinal canal. It is easily distinguished from its surroundings, appearing as a white opaque body about 0.7 mm. in length.

The body of the parasite is composed of an exceedingly large deutomerite (Pl. III, Fig. 37, *deu'mer*), containing the nucleus in its anterior portion, and a small protomerite (*pr'mer*), which bears an epimerite (*e'mer*).

Its surface is covered with fine longitudinal striations (Pl. III, Figs. 37–39) due to longitudinal folds in the cortical layer of the body (Pl. III, Figs. 41, 42). On focusing just below the surface, cross striations can occasionally be seen (Pl. III, Fig. 37); they are due to circular muscular fibres. In optical longitudinal sections of the parasite they are seen along its edges as black dots (Pl. III, Figs. 37, 38).

The outer cuticular wall appears very thick in longitudinal sections (Pl. III, Fig. 40); but this appearance is misleading, as cross sections of the parasite (Pl. III, Figs. 41, 42) clearly show. It is produced by the peculiar relation of the circular muscle fibres to the relatively thin cuticular covering and to the cortical portion of the parenchyma, which lies immediately below the cuticula. At short and tolerably regular intervals the circular contractile fibres come nearer to the deep surface of the cuticula and apparently have here a closer connection with that structure than over the intervening regions. The result is that the subcuticular layer of the parenchyma is thrown into longitudinal ridges, separated by sharp, deep furrows. The height of the ridges and the narrowness of their bases are to a certain extent dependent on the degree of contraction of the muscular fibres. The more the muscles shorten, the thinner and higher are the ridges. These ridges are so numerous and

close-set, and of so nearly uniform height, that in longitudinal sections, optical or actual, they have the effect, especially with low powers, of a very thick cuticular covering, as thick as the ridges are high. But the real thickness of the cuticula is only a very small fractional part of the height of the ridges, and is therefore many times less than the apparent thickness of the cuticula. That this relation of cuticula and contractile fibres is not an accidental or variable one is shown by the fact that the cuticula is not of uniform thickness, but presents at regular intervals, strictly correlated with its relation to the muscle fibres, longitudinal thickenings. These thickenings correspond with the crests of the ridges, and the middle of each thickening is raised into a sharp ridge (Pl. III, *cul*, Figs. 41, 42). On focusing with a high power a little above the level of the bottom of the furrows, the surface of the gregarine appears striated longitudinally (Pl. III, Fig. 43), granular stripes alternating with narrower clear ones. The clear stripes are caused by the depressions between the ridges; the darker granular stripes are optical longitudinal sections of the subcuticular substance of the ridges. At a slightly deeper focusing are seen fine, parallel, transverse markings much nearer together than the longitudinal stripes; these are due to the very fine, highly refractive circular muscle fibres (Pl. III, *mu*, Fig. 43). In cross sections these circular fibres (Pl. III, Figs. 41, 42, *mu*) can be seen running entirely around the parasite, stretching in succession from the cuticula at the bottom of one furrow to that at the bottom of the next. When the muscles are relaxed the ridges become low, rounded, and blunt (Pl. III, Fig. 41); but upon the contraction of the muscles the furrows become narrower and deeper, and the ridges higher and more pointed (Pl. III, Fig. 42).

The protomerite of this gregarine has the form of a biconvex lens set in a corresponding depression of the anterior end of the deutomerite (Pl. III, Figs. 44, 46, *pr'mer*). It is composed of a very compact and finely granular protoplasm. The anterior portion is slightly denser and more deeply stained than the posterior half. From the centre of its anterior surface the epimerite (Pl. III, Figs. 44, 46, *e'mer*) arises as a conical structure tapering off at its anterior end into a long filament (*fil*).



On removing the gregarine from the host the epimerite is generally torn off, remaining behind fastened to the intestine.

The filament being, so far as discovered, a simple structure unprovided with hooks, probably maintains its hold by a more or less sinuous course in the intestinal cells of the host. When the epimerite is not torn off, it invariably carries with it an irregular mass of protoplasm, part of the intestinal cell of the host in which it had anchored itself (Pl. III, *pr'pl*, Figs. 44, 46).

The conical base of the epimerite is apparently continuous with the contents of the anterior portion of the protomerite, for there is an orifice through the cuticular wall of the protomerite, and the base of the epimerite is composed of protoplasm very similar to that of the protomerite. The orifice is made very distinct by a considerable thickening of the cuticula at its margin (Pl. III, Figs. 44, 46, *fas*). In the cases where the epimerite has been torn off, the cuticula immediately surrounding the orifice appears pulled out a little by the strain of the rupture, thus leaving a cup-like depression at the anterior end of the protomerite (Pl. III, Fig. 48). Longitudinal sections through this cup show that the cuticular thickening just alluded to bends over its rim and extends about half-way down into the bowl (Pl. III, Fig. 45, *fas*). Cross sections through this region in the case of individuals retaining the epimerite show a row of teeth-like processes (*de*) extending inwards from the margin of the cup (Pl. III, Fig. 47). These project radially toward the axis of the epimerite, — I think from the lowermost portion of the cuticular band which bounds the orifice, — and perhaps serve to hold the epimerite more securely to the protomerite.

The parallel longitudinal ridges of the body wall of the gregarine, on reaching the protomerite, converge towards the cuticular ring surrounding the cup (Pl. III, Fig. 47); they become narrower and lower as they approach the ring, until at length they are so minute that in sections cutting across them (Pl. III, Fig. 49, *crs*) they resemble short cilia. The cuticular crests run up almost to the ring, where they terminate in a slight thickening (Pl. III, Fig. 48, *cul*).

The deutomerite is unusually large, as we have seen. It is composed of a very loose and highly vacuolated protoplasmic network, which does not stain.

The large nucleus (Pl. III, Fig. 46, *nl*) is usually situated, as I have said, in the anterior portion of the deutomerite; in living specimens, however, I have seen it move half the length of the animal. It generally contains several nucleoli, and in some of these, in addition to two or three vacuoles, I have noticed one or more very dark bodies.

In one case I discovered a second nucleus in the posterior half of the animal (Pl. III, Fig. 38). Here the deutomerite was slightly constricted just in front of the posterior nucleus; this certainly suggests preparation for division, and together with the condition shown in Pl. III, Fig. 39, which looks like a recently divided individual, makes it seem probable that division is one of the normal modes of reproduction with this gregarine.

I have found that this gregarine will live for seven or eight hours in sea water after being forcibly removed from the host. In watching the living specimens my attention was attracted by the very interesting movements made by the animal, which reveal a surprising amount of strength and a most remarkable manner of locomotion.

Most of the movements can be readily accounted for by contractions of the cortical portion of the parenchyma or the circular muscular fibres. Thus the movement in the direction indicated by the arrow in Pl. III, Fig. 50, or from the condition of  $\alpha$  to condition of  $\beta$  in Pl. III, Fig. 51, is undoubtedly due to the contraction of the cortex on the convex side of the body. The creases in the cortex (Pl. III, Fig. 50, *rug*) are due to the contraction of some of the circular muscle fibres; I believe they divide off successive areas of longitudinal contraction.

This gregarine is able to obtain a firm hold on smooth surfaces by using its anterior end as a sucker. On keeping a few of them for a time in a watch glass some have fastened themselves in this manner so tightly to the bottom that they could not be removed without tearing them to pieces. Pl. III, Fig. 53 shows the appearance of the anterior end when fastened in this way to a cover glass.

The strength of the gregarine was well shown in one case where, after having fastened its head to the slide, it raised the middle portion of its body and turned it through an arc of  $180^\circ$ , like the bail of a bucket, its head and the tip of its tail alone resting on the slide during the process (Pl. III, Fig. 52). From the fact that the general shape of the animal did not change during the operation, it is probable that the entire movement was caused by the contraction of a very small portion of the body wall. The area of contraction must have been limited to the region of *a* (Pl. III, Fig. 52), since the head alone was fixed, the tail being free and changing its position slightly.

The most interesting movement observed, however, is that of locomotion. It is a "very slow movement of translation in a straight line" without any apparent contraction of the walls of the body. It is probably caused by a very slight undulatory motion of the under surface of the animal.

My material, obtained at Woods Holl, was all collected in the months of July and August. Perhaps this is the reason why I have not found the encysted stage of this gregarine.

This work was begun in the summer of 1891 at the suggestion and under the direction of Dr. C. O. Whitman; it was completed during the past winter under the very kind and careful supervision of Dr. E. L. Mark in the zoölogical laboratory of Harvard College. I wish to express a deep feeling of indebtedness to my instructors for whatever there may be of value in this paper.

CAMBRIDGE, MASS.,  
May 21, 1895.



## EXPLANATION OF PLATES.

All the figures were outlined with an Abbé camera lucida. The very high magnifications were obtained with a Zeiss 1.5 mm. apochromatic homogeneous immersion objective and compensating oculars nos. 4, 6, and 12.

## ABBREVIATIONS.

<i>cav</i> ,	cavity.	<i>gran</i> ,	granules.
<i>chr</i> ,	chromatin.	<i>mb</i> ,	membrane.
<i>cps</i> ,	capsule.	<i>mu</i> ,	muscle.
<i>cp. sing</i> ,	blood corpuscles.	<i>nl</i> ,	nucleus.
<i>crs</i> ,	crest of ridge.	<i>pr'mer</i> ,	protomerite.
<i>cta</i> ,	cuticula.	<i>pr'pl</i> ,	protoplasm.
<i>cul</i> ,	ridge.	<i>rtl</i> ,	reticulum.
<i>cys</i> ,	cyst.	<i>rug</i> ,	crease.
<i>de</i> ,	teeth.	<i>spo</i> ,	spores.
<i>dep</i> ,	depression.	<i>spo'go</i> ,	} sporogonia.
<i>deu'mer</i> ,	deutomerite.	<i>spo'go'</i> ,	
<i>e'mer</i> ,	epimerite.	<i>spo'go''</i> ,	
<i>fus</i> ,	band.	<i>sul</i> ,	furrow.
<i>fil</i> ,	filament.	<i>vac</i> ,	vacuole.



## EXPLANATION OF PLATE I.

FIG. 1. Cross section of *Clymenella torquata*, showing sections of eighteen gregarine cysts.  $\times 40$ .

*cys*, cysts before the beginning of spore formation.

FIG. 2. Mature living cyst illuminated from above.  $\times 136$ .

FIG. 3. Conjugating gregarines drawn from a living specimen.  $\times 136$ .

FIG. 4. Gregarine just after encystment, drawn from living specimen.  $\times 136$ .

FIG. 5. Early stage in spore formation, drawn from parasite.  $\times 136$ .

FIG. 6. Section of a gregarine showing the breaking up of the nucleus before encystment.  $\times 350$ .

*cav*, cavity caused by the shrinking of the protoplasm away from the surrounding tissue of the host.

*pr'pl'*, granular protoplasm.

*pr'pl''*, frothy or vacuolated protoplasm.

FIG. 7. Section of gregarine, imbedded in tissue of host, after disappearance of nucleus and before the beginning of sporulation.  $\times 350$ .

$\alpha, \beta, \gamma, \delta$ , spaces occupied by other cysts.

FIG. 8. A portion of the central mass of protoplasm shown in Fig. 9 highly magnified.  $\times 3100$ .

FIG. 9. Section of a cyst showing the formation of sporogonia and the condition of the central mass of protoplasm.  $\times 350$ .

FIG. 10. Another section of the same cyst.  $\times 350$ .

FIG. 11. Portion of the same cyst more highly magnified, showing structure of recently formed sporogonia, their manner of formation, and the condition of the protoplasm.  $\times 1540$ .

*rtl*, protoplasmic network.

FIG. 12. Section of cyst in a later stage of the formation of sporogonia.  $\times 230$ .

*cys*, wall of cyst.

*a*, former position of wall of cyst, determined by surrounding tissue of host.

*rtl*, fine filamentous network.

FIG. 13. Part of central mass of protoplasm, shown in Fig. 12, more highly magnified.  $\times 1100$ .

*spo'go*, recently formed sporogonia.

*spo'go'*, sporogonia not yet fully formed.

*chr*, mass of chromatin granules.

FIG. 14. Sporogonia from same cyst.  $\times 3100$ .

FIG. 15. Section of cyst when spore formation is almost completed.  $\times 400$ .

*pr'pl*, protoplasm not yet converted into sporogonia.

*spo'go*, sporogonia with eight nuclei.

*spo'go''*, a sporogonium after the first division of the nucleus.

*mb*, exceedingly thin transparent membrane.











## EXPLANATION OF PLATE II.

FIG. 16. Section of a cyst containing sporogonia in several stages of development.  $\times 400$ .

*cys*, the shrivelled cyst.

$\beta$ , former position of the wall of the cyst as determined by the surrounding tissue.

$\alpha$ , see explanation of Fig. 17.

FIG. 17. Group of sporogonia, seen at  $\alpha$  in Fig. 16, more highly magnified.  $\times 1540$ .

FIGS. 18, 19. Recently formed sporogonia killed with Perenyi's fluid stained with borax carmine.  $\times 3100$ .

FIG. 20. Sporogonia after the first division of the nucleus; treatment of the preparation the same as that of Fig. 18.  $\times 3100$ .

$\alpha$ , immediately after the division of the nucleus has taken place.

$\beta$ , a little later stage than that shown at  $\alpha$ .

FIG. 21. Sporogonium after the second division of the nucleus; otherwise same as FIG. 20.  $\times 3100$ .

FIG. 22. Sporocyst showing early stage in the development of the spores.  $\times 3100$ . Two sac-like envelopes already formed; the outer one ( $\alpha$ ) the capsule; the inner one ( $\beta$ ) the cyst.

$\alpha$ , thickening of the cuticula at the mouth of the capsule.

$\beta$ , narrow neck of inner cyst.

FIG. 23. Sporocyst flattened somewhat by pressure of cover glass. Spores in a little more advanced stage than in Fig. 22.  $\times 3100$ .

FIG. 24. Cross section through the middle of sporocyst and contents in the condition represented in Fig. 23.  $\times 3100$ .

FIG. 25. Cross section of another sporocyst of the same age as Fig. 24.

FIG. 26. Inner cyst of sporocyst; spores not yet fully matured; four of them have been squeezed out.  $\times 3100$ .

*pppl*, thread-like portions of contents of cyst not employed in formation of spores.

FIG. 27. Two spores forced out of cyst.  $\times 3100$ .

$\alpha$ , clear area due to shrinkage of nucleus.

FIG. 28. Two abnormal spores.  $\times 3100$ .

FIG. 29. Mature sporocyst containing seven spores, one in process of emerging: the eighth spore had already escaped.

$\alpha$ , thickening of cuticula around the opening at upper end of capsule.

FIG. 30. Mature sporocyst with seven spores, three of them leaving the cyst. Probably one spore had already entirely escaped.  $\times 3100$ .

FIG. 31. Mature sporocyst showing a frequent arrangement of spores in cyst. Stained with Bismarck brown.  $\times 3100$ .

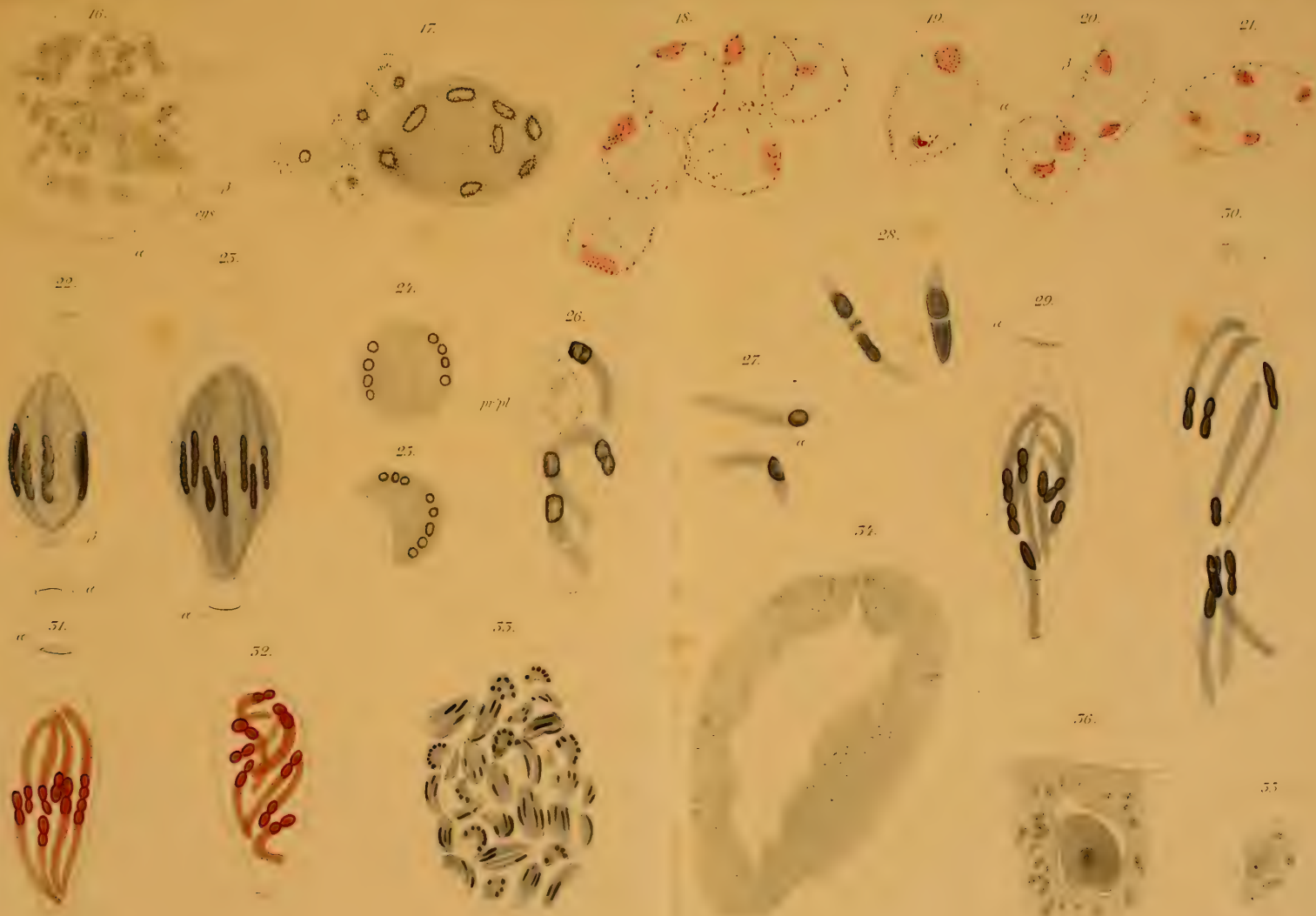
FIG. 32. Inner cyst showing irregular arrangement of the eight spores, some of which are considerably curved. Stained in Bismarck brown.  $\times 3100$ .

FIG. 33. Portion of a section through a gregarine cyst showing sections through sporocysts in various directions.  $\times 1100$ .

FIG. 34. Cross section of intestine of *Clymenella torquata* showing large amoeboid cells, possibly early amoeba-like stage of this gregarine.  $\times 77$ .

FIG. 35. Amoeboid cell in wall of intestine near its outer body-cavity surface, more highly magnified.  $\times 350$ .

FIG. 36. Another amoeboid cell of larger size and more deeply imbedded in the wall of the intestine.  $\times 350$ .





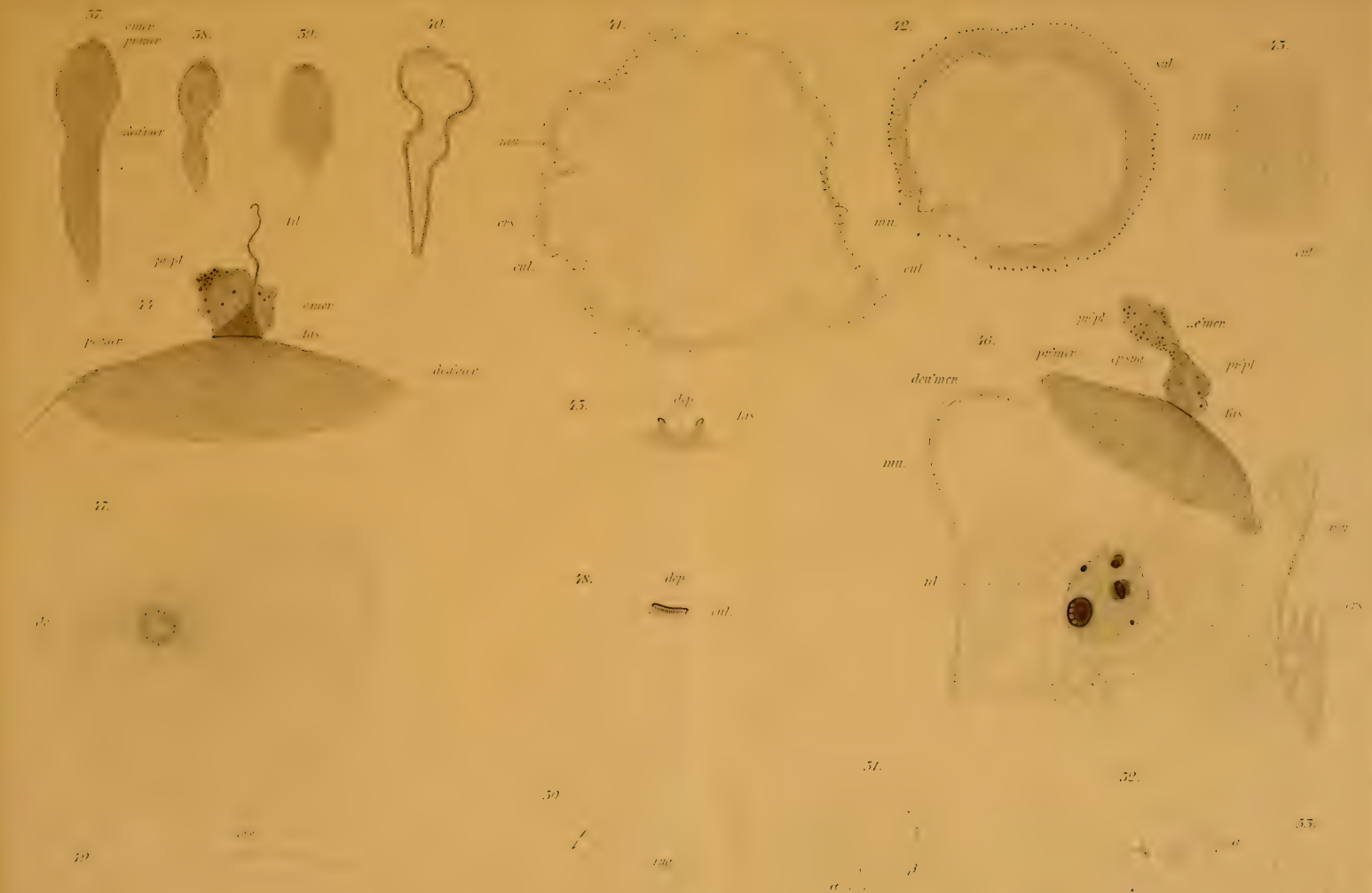




## EXPLANATION OF PLATE III.

*Gregarine parasitic in Rhyncobolus Americanus.*

- FIG. 37. Normal appearance of a detached gregarine.  $\times 77$ .
- FIG. 38. Individual with two nuclei, perhaps about to divide.  $\times 77$ .
- FIG. 39. Gregarine, possibly just after division has taken place.  $\times 77$ .
- FIG. 40. Longitudinal section showing apparently a thick cuticular wall.  $\times 187$ .
- FIG. 41. Cross section through the middle of the parasite showing the actual thickness of the cuticula and the longitudinal folds into which it is thrown.  $\times 1100$ .
- mu*, circular muscle fibres, in this case somewhat relaxed.
- FIG. 42. Cross section, the circular muscular fibres (*mu*) contracted.  $\times 1100$ .
- FIG. 43. Surface view, focusing at the level of the bottom of the furrows (*sul*), and showing the circular muscle fibres (*mu*).  $\times 1100$ .
- FIG. 44. Section through the anterior end of the gregarine.  $\times 1100$ . The protomerite (*pr'mer*) is seen to be filled with finely granular compact protoplasm. The conical epimerite (*e'mer*), terminates in a long slender filament (*fil*), and is enveloped in a loose mass of protoplasmic substance (*pr'pl*), probably derived from the intestinal epithelium of the host.
- fas*, thickened cuticular band forming a rim around the cup-shaped depression. This is left behind whenever the epimerite is torn away from the protomerite.
- FIG. 45. Section through the cup-shaped depression at the anterior end of the gregarine.  $\times 1100$ .
- FIG. 46. Section through anterior end of gregarine.  $\times 900$ .
- cp. sng*, blood corpuscles scattered in the protoplasm from host.
- FIG. 47. Surface view of anterior tip of gregarine. The most of the epimerite has been cut away. Tooth-like processes (*dc*) can be seen projecting radially into the base of the epimerite, which remains in the cup-shaped depression.  $\times 1100$ .
- FIG. 48. Side view of the cup-shaped depression and the anterior tip of the gregarine.  $\times 1100$ .
- FIG. 49. Section through the protomerite. In the middle of the figure the radial ridges (*crs*) are cut crosswise.  $\times 1100$ .
- FIGS. 50-52. Different forms and positions assumed by living gregarines.
- FIG. 53. Outline of the head of a living gregarine when pressed against the cover glass and used as a sucker.







## ON THE OSTEOLOGY AND RELATIONSHIPS OF PROTOSTEGA.

E. C. CASE.

### INTRODUCTION.

THE systematic arrangement of the sea turtles, or *Pinnata*, has long been a mooted point among zoölogists. Prior to the year 1870 there was practical unanimity in placing *Dermochelys* near the members of the *Cheloniidae*. In the year 1871 Cope (1) separated these forms, and placed *Dermochelys* in a distinct group, *Athecae*, opposed to and of equal rank with the *Cryptodira* and *Pleurodira*. One year later, in discussing the genus *Protostega* (2) he placed it "near the *Sphargidae* in the sub-order *Athecae*, and in some points to be approximated to the *Cheloniidae*." In 1875 he established the family *Protostegidae* (3), a name he had used two years earlier (4).

The group *Athecae* was apparently accepted by Gervais in his description of *Dermochelys* (*Sphargis*) (5), and the separation of the group was recognized by Seeley (6), who in 1880 placed *Dermochelys* in a group *Dermatochelyidae*, of equal rank and value with two opposing groups, *Peltochelyidae* and *Aspidochelyidae*.

Döderlein (7) accepted Cope's classification with the addition of the group *Trionychoidea*, and this group was subsequently adopted by Cope (11). Böttger (29) in 1895 recognized the *Athecae*. Dollo (8) in 1886 published a paper in which he raised the value of Cope's group *Athecae* by placing it in opposition to all the remaining *Testudines* grouped under the name *Thecophora*. This idea he subsequently defended in two papers which appeared in 1887 (9) and 1888 (10).

Dollo was supported by Smith-Woodward (12), Bernard (18), and by Boulenger, both alone (13) and in collaboration with Günther (14). These later authors substituted, as did Lydekker, (15, 16) the name *Testudinata* for Dollo's *Thecophora*.

In 1873 Rütimeyer (17) disregarded Cope's classification of two years previous, and placed *Dermochelys* among the *Pinnata*.

In 1886, the same year as Dollo's first paper, Baur published a note in the *Zoologischer Anzeiger* (19), in which he claimed that the separation of *Dermochelys* from the *Cheloniidae* was absolutely artificial. He maintained his position in papers appearing at intervals from 1888 to 1893 (20-26).

Zittel (27) in his text-book, and later Dames (28), disregarded the group *Athesae*, the former considering the *Dermochelyidae* as a family of the *Cryptodira*.

There are then at present three views as to the position of *Dermochelys*. (1) It is closely related to the *Cheloniidae*, being merely a specialized form. (2) It is the sole representative of a group equal in rank to all the remaining *Testudines*. (3) It is the representative of a group of equal rank with the *Trionychoidea*, *Cryptodira*, and *Pleurodira*.<sup>1</sup>

Paleontology alone can decide which of these theories is correct, and, fortunately, a turtle from the middle cretaceous of Kansas, *Protostega* Cope, is known, which from its intermediate form affords most valuable evidence in completing the phylogeny of the existing sea turtles. This paper is concerned in describing additional remains of this animal, and discussing its relationships to allied forms.

#### *Description of Protostega and Comparison with Related Forms.*

The material used in the following descriptions of *Protostega* consists of two specimens, both from the Niobrara cretaceous of Kansas. The first and larger specimen comprises the

<sup>1</sup> It may be of interest to give here the synonymy of *Dermochelys*.

1816: *Dermochelys*, Blainville, Bull. des Sciences par la Société philomatique de Paris, année 1816, p. 119 (wrongly printed 111). (See Baur's discussion of the names *Dermochelys*, *Dermatochelys*, and *Sphargis*, Zool. Anzeiger, no. 270, 1888.)

1820: *Sphargis*, Merrem, Versuch eines Systems der Amphibien, p. 19.

1822: *Coriudo*, Fleming, Philosophy of Zoölogy, vol. ii, p. 271.

1828: *Scytina*, Wagler, Oken Isis, 1828, part 2, p. 861.

1829: *Dermatochelys*, Wagler, Nat. Syst. Amphib., S. 133.

1832: *Chelyra*, Rafinesque, Atlantic Journal and Friend of Knowledge, vol. i, no. 2, Philadelphia, Summer of 1832, p. 62.



greater part of the plastron and limbs of a very large individual. The bones are in excellent condition, and the sutures very distinct. There are present the hyoplastra of both sides, the hypoplastron of the left side, and weathered fragments of that of the right, the xiphiplastron of the left side, and the distal end of that of the right, the nuchal and eight peripherals determinable as belonging in series on the left side, and fragments of others, the humeri, scapulae, and coracoids of both sides, and the proximal ends of the radius and ulna (?). The femur of the left side, the pubis and ilium of the same side with the distal end of the ischium, the ischium of the right side with the distal ends of the ecto- and ento-pubis. Several incomplete ribs, Pl. IV.

The second and smaller specimen preserves the humerus, scapula, and coracoid of the left side, a singularly complete pelvis, and some incomplete ribs. The greater part of the skull is in fairly good condition, showing the basi-, supra-, and ex-occipitals, the paroccipital, quadrates, petrosals, quadrato-jugal, and squamosal, the basisphenoid, pterygoids, and palatines, and the almost perfect lower jaw.

*Skull.* — The *supraoccipital* was greatly flattened from side to side in the process of fossilization. The ridge forming the upper edge of the bone slants downward and backward, its distal part is incomplete, though apparently only a small part has been lost. The superior-anterior portion bears a narrow face which slants downward and forward for a considerable distance. These regions are almost identical with the same regions in the *Cheloniidae*, and are widely different from *Dermochelys*, where the upper edge of the supraoccipital is almost horizontal, and is broad and rounded. The face on the anterior aspect is broad, horseshoe-shaped, and almost vertical.

The region bearing the articular faces for the exoccipitals, petrosals, and paroccipitals is moderately expanded and is quite solid, showing the absence of any great amount of intercalated cartilage, such as occurs in *Dermochelys*, where the articular faces are represented by rugose pits, and are not distinguishable one from the other. The articular faces for the petrosal and paroccipital meet on the summit of a ridge running outwardly from the external edge of the anterior semicircular canal.

This canal is represented by a deep triangular pit, no part of which is covered by processes from the sides.

This condition of the semicircular canal is exactly that of the *Cheloniidae*, and differs widely from *Dermochelys*, in which it is roofed by three distinct processes meeting in the middle and leaving three foramina for communication with the other semicircular canals (Pl. V, Fig. 1).

The *exoccipital* of the left side is badly crushed, but is still in sutural union with the *paroccipital* of the same side, and the two are in connection with the *supraoccipital*. The *exoccipital* of the right side is separate and almost perfect. The ascending process for the *supraoccipital* is short and strong. The descending process is short, and did not reach connection with the *pterygoid*. The articular face for the *paroccipital* is deeply excavated. The condylar foramen is near the condylar portion, which is well ossified and free from osseous connection with the same region of the *basioccipital*. This complete and separate ossification of the condylar region is a point of decided difference between the *Cheloniidae* and *Dermochelys*; in the latter the region is almost entirely cartilaginous, and the three bones are weakly anchylosed in old specimens (Pl. V, Fig. 2).

The *basioccipital* is a comparatively broad and short bone with well-ossified condylar portion and strong lateral processes terminating in rugose extremities which extended between the *pterygoids* and the *exoccipitals*. The under surface is nearly smooth, and lies in the plane of the horizontal axis of the skull. The articulation for the *basisphenoid* is confined to its anterior end.

In every particular but that of the ossified condylar portion the *basioccipital* of *Protostega* agrees with *Dermochelys*. In the *Cheloniidae* the lateral processes are small, and the *pterygoid* articulates with the *exoccipital*; this causes the *basioccipital* to lie largely between the *exoccipitals*, instead of below them as in *Dermochelys*. The inferior surface of the *basioccipital* in the *Cheloniidae* varies from being almost horizontal to being inclined steeply downward and forward, and the *basisphenoid* may cover it far back towards its middle (Pl. V, Fig. 3; *a*, from above; *b*, from below).

The *petrosals* are both present in a very perfect condition. They are roughly triangular, and have a strong ridge, partly due to pressure, on the external surface. The external face also shows a deep excavation corresponding to a similar excavation on the antero-interior portion of the quadrate. The union of the sides of these two excavations forms the foramen for the external carotid artery, and probably excluded the paroccipital from any part of the foramen.

The external semicircular canal is represented by a deep pit bridged in its antero-superior region by a bony bar reaching from side to side, and leaving in front of it a foramen for communication with the anterior canal.

The articular face for the basisphenoid is broad and strong.

The formation of the carotid foramen, as well as the nature of the semicircular canal, is typically that of the *Cheloniidae*. In *Dermochelys* the paroccipital takes large part in the formation of the foramen, and the pit in the petrosal is entirely free from any bony processes (Pl. V, Fig. 4; *a*, from within; *b*, from without).

The *paroccipital* of the right side is present in almost perfect condition. The bone is elongated and reaches connection with the squamosal, a character which never appears in *Dermochelys*. The posterior or external half of the posterior semicircular canal is roofed by a bony process pierced by two foramina which communicate with the other canals. This character of the posterior semicircular canal appears in the *Cheloniidae*, and is very different from *Dermochelys*, where there is a single bony process which does not reach entirely across the canal (Pl. V, Fig. 5).

The *basisphenoid* is badly crushed, but retains somewhat its original character. It is almost round in outline, with a smooth under surface. The upper face is traversed in a longitudinal direction by two deep grooves. There is no trace of an anteriorly extending rostrum on the thickened anterior end. The smooth under surface appearing largely on the base of the skull, with no trace of a ridge where it meets the basioccipital, is similar to the condition found in *Dermochelys*, though in that genus the basisphenoid takes much larger part in the



formation of the base of the skull, and separates the pterygoids for a greater distance than it did in *Protostega*.

The *quadrates* are present in excellent condition; they are still connected with the pterygoids, which are in turn united with the imperfect palatines. The articular face for the quadrato-jugal is strongly developed, and stands on the summit of a prominent ridge. The anterior edge is thin and rounded in outline; near its inferior portion there is developed a short, stout process, which fits into a deep groove on the external face of the pterygoid. This process gave attachment to the columellar plates or the cartilage of its lower end. This strong process of the quadrate is present in *Dermochelys*; in the *Cheloniidae* it is very slender, and may even be absent as in *Lepidochelys*.

Near the anterior inferior portion of the inner side lies the groove which in connection with the groove on the petrosal forms the foramen for the external carotid artery.

The condylar face is divided into two parts; one, the posterior, looking slightly upward and backward; the other saddle-shaped, and looking almost directly downward. The upper posterior portion of the bone shows a strong face for attachment with the squamosal. The most distinctive feature of the bone, and one which is shared with none of the other sea turtles as far as observed, is the manner of attachment to the pterygoid; the posterior portion of that bone reaches almost to the condylar face, instead of being separated from it by a considerable space. The quadrate stood at almost a right angle to the pterygoid (Pl. V, Fig. 6).

The *stapes* was comparatively a very large bone. The distal end of one preserved in the stapelial notch of the right quadrate is larger than the same bone in a skull of *Dermochelys* twice as large as the skull here described.

The *pterygoids* of both sides are present in fairly good condition, the internal edges only being broken and crushed. They are long, slender bones with rounded external edges, decidedly concave external margins, and with no trace of an ectopterygoid process. The posterior portion articulates far down on the quadrate as described, and the posterior external face shows a

deep groove running forward and upward which receives the epipterygoid process from the quadrate.

The posterior half is not perforated by a branch of the carotid artery as in the *Cheloniidae*, nor does any foramen for this artery appear on the back of the skull as in that family. In these points of difference from the *Cheloniidae*, and in the fact previously mentioned that it is separated from the exoccipitals by the lateral processes of the basioccipital, the pterygoid agrees with *Dermochelys* (Pl. V, Fig. 6).

The *palatines* are present in an imperfect condition. The anterior and interior portions are gone, and the whole bone is distorted by pressure; enough remains, however, to show that there were deep choanae located far forwards which were not roofed by the vomer and palatines. This condition of the internal nares is largely that of *Dermochelys*, in which the choanae are far forward, and are not roofed by the palatines and vomer. The articulation with the maxillaries was by a deep, elongated, triangular region, as in the typical *Cheloniidae* (Pl. V, Fig. 6).

The *vomer* is present in a fragmentary condition. It did not have a process descending between the palatines, and helping to roof the choanae, as in the *Cheloniidae*.

The *quadrato-jugal* of the right side is triangular in general outline. The posterior edge is concave, and the whole bone is convex from above downwards. The superior edge is narrow, and there is no prolongation of the antero-inferior portion, as in *Dermochelys* (Pl. V, Fig. 7).

The *squamosal* of the right side shows a broad concave surface for the upper end of the quadrate. The posterior inferior portion shows no groove as in the *Cheloniidae*. The anterior portion is thin and expanded.

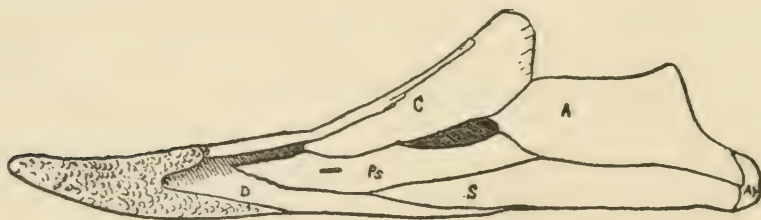
The *mandible* is present in a singularly perfect condition, the only parts injured being the posterior portions of the complementaries. It is figured in Pl. V, Fig. 8. The whole jaw resembles very much that of the *Cheloniidae*.

The *dentary* is broad from above downwards, with the upper surface slightly concave in the region of the symphysis, and marked by deep pits. The symphysis is broadly triangular, it

extends farther back on the lower surface than on the upper, and its posterior part is marked by a deep pit. The dentary reaches very nearly to the posterior extremity of the mandible, covering in large part the complementary and the surangular.

The *complementary* is present and complete; it is largely covered externally by the dentary, which also overlaps the superior margin, and appears on the supero-internal edge. The posterior end rises rather high, and terminates abruptly. Its postero-inferior angle articulates with the angular, and forms a bridge over the cavity in the ramus.

The *presplenic*. Dr. Baur has drawn my attention to the fact that the element described by him (44) in the jaw of certain pleurodiran turtles (the group *Chelyoidea*), in the *Crocodylia*, and in the *Lacertilia* as the *presplenic*, also occurs in *Protostega* and *Toxochelys*. It is an elongated element articulating with the angular and splenic behind, the dentary below, and the coronary above, occupying the same position as in the *Chelyoidea*, but extending far forward, and covering the groove on the internal surface of the ramus except in its anterior extent. It is pierced by a foramen near its anterior edge. Its general form and relations are shown in the accompanying figure.



The *splenic* is distinct from the angular, and appears as a long, splint-like bone reaching forward towards the symphysis. It appears largely on the under surface of the mandible.

The *angular* is short and broad; it sends a process upwards to meet the complementary. The posterior end shows a large face concave from above downwards, and looking almost directly backwards. It rises above the surangular, concealing it on the interior aspect.

The *surangular* is broad and short, joining the complementary by its antero-superior portion. The posterior portion bears an articular face, concave from above downwards, and looking slightly inwards; the external margin of this face ends on a thin ridge.

The *articular* is well ossified. Its articular face, slightly saddle-shaped, looks backward and upward. The postero-inferior part is rounded, and shows largely on the inferior surface of the mandible. There is no process on the infero-internal portion of the articular, as in the *Cheloniidae*, and this allows the condylar face of the angular to curve towards the articular at the bottom instead of bending toward the median line of the jaw.

*Plastron*. — The *hyoplastron* is a large, heavy plate, thickened in the middle and becoming thin towards the edges, which are extended into long, slender, radiating processes. It is roughly triangular in shape. The antero-internal portion is bent slightly inward, and carried as a strong wing toward the median line, where the terminal processes meet those of the same of the opposite side. The antero-external edge is smooth, free from processes, and concave. The external edge is thickened, and the terminal processes are comparatively short and strong.

The articulation with the hypoplastron was by small and numerous closely interlocking processes amounting almost to a sutural union. The connection occupied nearly half the posterior edge of the bone (Pl. IV).

The *hypoplastron* is of almost equal size with the hyoplastron. Its general shape is more nearly round, it is furnished with processes on the edges, and those on the posterior internal edge meet those from the same bones of the opposite side. The posterior edge was furnished with two long processes diverging posteriorly, between which the xiphiplastron articulated.

The appended measurements will give an idea of the size of these plates, though the loss of the distal ends of the processes makes exact measurements impossible (Pl. IV).

Greatest length hyoplastron	. . . . .	.649 meters
" breadth "	. . . . .	.446 "
" length hypoplastron	. . . . .	.578 "
" breadth "	. . . . .	.377 "



The *xiphiplastron* is an elongated bone articulating by an interlocking joint between the posterior processes of the hypoplastron. It differs from all sea turtles in the peculiar bending of the bone near its middle; originally directed inwards and backwards, it changes its course abruptly, and is directed inwardly at almost a right angle to its original course. It articulated strongly with the xiphiplastron of the opposite side (Pl. IV and Pl. V, Fig. 9).

There is nothing preserved of the ento- or epi-plastron.

The general shape of the plastron was broadly ovate with the posterior end truncate. The fontanelle was diamond-shaped, and bridged at its anterior and posterior extremities by the lateral processes from the different plates.

The plastron stands midway between that of *Dermochelys* and that of the *Cheloniidae*. In *Dermochelys* the union of the hyo- and hypo-plastra is by the overlapping of the extremities of the slender bones which have lost their radial processes. The xiphiplastron is straight, and articulates by overlapping with the hypoplastron.

In *Protosphargis* Cap., the plastral bones are more robust than in *Dermochelys*. The marginal processes are retained to some extent, and the hyo- and hypo-plastra articulate by the interlocking of a few digital processes. The xiphiplastron is straight, and articulates with the hypoplastron by overlapping.

The *Cheloniidae* have a broad sutural union between the two plates of the plastron. The marginal processes are confined to the distal ends of the bones, leaving the edges near the suture round and smooth. An approximation to this state can be noticed in *Protostega*, where the marginal processes near the union of the plates are shorter than those on the ends. The nature of the processes varies among the members of the *Cheloniidae*. In *Lepidochelys kempi* Garm. they are numerous and irregular, standing on a base that leaves the main body of the plate in a curve, thus forming an oval or rounded fontanelle. In *Chelonia* there are generally only three processes on the hypoplastron, two of which project from the body of the bone at right angles, and meet across the squarish fontanelle, while the other passes obliquely forward toward the epiplastron.

The series with *Dermochelys* at one end, and *Chelonia* at the other, is marked by a constant variation in the size of the plastral elements, the nature of union of the bones, and the presence and position of the marginal processes.

*Carapace.* — The *ribs* are present in fragmentary condition in both specimens. The head was well developed and separated from the costal plate, the proximal end of which was expanded and produced into slender digitations. Examination of a specimen of *Chelonia mydas* shows that the distance from the point of union of the rib head with the costal plate to the vertebral articulation is greater than the distance from the same point to the neural edge of the costal plate. In *Protostega*, as these specimens show, the opposite is true even when as in this case the measurements are carried only to the broken ends of the digitations. This shows that there was proportionally less room between the proximal ends of the costal plates in *Protostega* than in the living sea turtles, and in all probability too little room to allow the presence of neurals. This supposition is further borne out by the digitated proximal ends of the ribs and the entire absence of anything that can be referred to neurals in the known specimens. The expansion of the ribs extends for about half their length (Pl. VI, Fig. 19).

The *nuchal plate* is very peculiar in form, resembling most nearly the nuchal of the soft-shelled turtles. In the present specimen the plate lies directly on the hyoplastra, having been crushed down on them, and has preserved them in their correct relative positions; it is thickened in the middle, becoming thin laterally, and expanded into broad wings. The distal ends of the wings are irregular in outline, and probably articulated weakly with the first peripheral. The upper surface shows two low rugose ridges which run from the center out into the wings, and there disappear. The anterior edge was concave, and beveled from above downward and backward. The posterior edge is continued into a long, slender process running back over the vertebral column. The posterior end of the process is broken off, but apparently only a small portion is missing. There is no process on the under side for articulation with the posterior cervicals (Pl. V, Fig. 10).

Following are some measurements of the nuchal plate.

Length from tip to tip of wings . . . . .	.599 meters
Length from middle of anterior edge to end of posterior process .168	"
Thickness in center . . . . .	.032 "
Breadth of left wing in broadest part . . . . .	.131 "

The *peripherals* are represented by eight from the left side, which are determinable as belonging in series, and several detached bones whose position is doubtful. The series extends from the second (?) to the ninth. The anterior, which probably joined the nuchal, is unfortunately lost. The second (?) peripheral is slender, concave on its outer edge, and bears no facet for a rib. It articulates strongly with the third. The third is strong and broad, and bears a deep pit for a rib near its external margin. The thickened external margin is turned slightly downward and inwards.

In the succeeding peripherals the length becomes greater than the breadth, and the external margin becomes acute. The turning in of the margin begun on the third becomes broader and broader, shoving the pit before it till in the posterior peripherals it occupies half the under surface of the bones, and its anterior edge underlies the centrally located pit. The inner edges of the peripherals are irregular, and extended into slender processes. Pl. V, Fig. 11, shows the upper surface of the fifth and sixth, and gives a good idea of the general shape of the peripherals and their strong articulation one with another. The following measurements are accurate in so far as the broken condition of the inner edge would permit.

Length second (?) peripheral . . . . .	.170 meters
Breadth " " . . . . .	.030 "
Length third " " . . . . .	.202 "
Breadth " " . . . . .	.118 "
Length fourth " " . . . . .	.180 "
Breadth " " . . . . .	.092 "

The condition of the carapace of *Protostega* as described above is heralded in the young of *Chelone Benstedii* Owen, where the costal plates taper from the proximal to the distal end, and in *Allopleuron* Baur (*Chelone Hoffmani* Gray), where the ribs have become very slender and the costalia short and

broad. In *Protosphargis* and *Dermochelys* the rib head is not covered by an expansion of the upper surface of the rib.

The loss of the neurals may find its initial step in the condition of *Eosphargis* (*Chelone gigas* Owen). There are in that genus, as described by Lydekker (30), six or seven large plates overlying the ribs; these were considered by Owen in the original description as neurals, but are considered by Lydekker as dermal scutes. It is difficult to see, however, whence the "median dorsal row of large carinated scutes" may have taken their origin if they are not neural plates which have lost connection with the vertebrae, and become laterally expanded so as to cover the ribs in part. This loss of connection between the neurals and vertebrae may be observed in the recent *Lepidochelys kempi* Garm., where 1, 2, 3, and also 8 are free (21).

The strong peripherals of *Protostega* were possibly present in *Eosphargis* (30), and peripherals have been observed in *Protosphargis* (31). They were very slender in *Protosphargis*, and were considered by the original describer as phalanges, but were later shown by Baur to be peripherals.

Most of the *Cheloniidae* have the typical number, eleven, but *Thalassochelys* and *Lepidochelys* have more, the number being varied by the introduction of one or two extra plates between the 1 and 3 (22).

The nuchal plate of *Protostega* differs widely from that of the living sea turtle, but in no point more widely than in the complete absence of the process on the under side for articulation with the last cervical. In *Osteopygis*, a sea turtle from the cretaceous, there is no trace of this process, but in *Lytoloma*, a form from the upper cretaceous and eocene, the eocene forms show the beginning of the process in a small tubercle (22).

The carapace of *Protostega* is now seen to be intermediate between the *Dermochelyiidae* and the *Cheloniidae*, with several primitive characters which are ancestral to both.

The *vertebrae* are represented by only two, from the caudal region. These are deeply concave in front, with the arch ossified with the centrum. The anterior zygapophyses extend well forward of the anterior edge of the centrum, and the top of the



arch is broad and rugose. There is a triangular articular face at the base of the arch on the anterior portion of the centrum. The description of the vertebrae from *Protostega* given by Cope (32) is here quoted to show their general nature.

He says of the vertebrae: "These have been recognized chiefly by their neural arches, which are separate. They are in form something like an X, the extremities of the limbs carrying the zygapophysial surfaces. The only point of contact with the centrum is a wide process, which stands beneath the anterior zygapophyses, and spreads out foot-like obliquely forward and outward to beyond the line of the anterior margin. Its surface extends nowhere posterior to the surface of the zygapophysis above it, but a little farther inward. Its outer margin rises ridge-like to the under side of the neural arch, and each one, forming a semicircle, forms the boundary of the neural, and turning outward, forms the *inner* boundary of the posterior or down-looking zygapophysis. The space between these apophyses is roofed over so as to produce a shallow zygantrum, which, however, only seems to roof over the deep emargination of the neural arch of the vertebra immediately following. The anterior zygapophyses are often broken away, so that the neurapophysial supports look like the missing pair, when the difficulty ensues that both pairs look downward. The top of the neural arch is, in two cases, broad and flat; in two others there is an obtuse keel.

"The centra, apart from their arches, are puzzling bodies, especially since in the present case they are somewhat flattened by pressure. They differ materially in size, one of them being twice the size of the others. The smaller ones are of the ball-and-socket type, and have a deep longitudinal groove on each side. The thickened portion of the centrum forms the inferior boundary of the pit groove, while a thinner portion, possibly a diapophysis, limits it above. It is, however, thin, and has no great length. There is no sign of chevron bones and articulation, so that these vertebrae may have been cervical. Their bodies are, however, shorter and wider than in those vertebrae of any known tortoise. A groove on the upper surface represents the neural canal, while a flat area on each side in front

supports the neuropophyses. The large centrum exhibits the superior groove and antero-lateral platform for support of the neural arch. One end is cupped obliquely, while the other is nearly plane, with the same obliquity and a slightly raised margin. Its outline is subtriangular. The lower side of this centrum possesses a short keel posteriorly. The sides exhibit no pit, but have a thin edge, which is concave behind the middle and then turned outward. I can see no articulation for a rib."

These vertebrae are stated by Cope to be most closely related to *Dermochelys*. Unfortunately, the material is too limited to admit any positive conclusions to be drawn as to the relationship of *Protostega*; but it is necessary here to note the close resemblance between the cervical vertebrae of *Dermochelys* and the *Cheloniidae*. Both have the strong articular process for the nuchal plate on the last cervical, and the articular faces between the 6 and 7 are plain.

*Limbs*.—The *humerus* is very broad and strong. The area for cartilaginous attachment on the mesial process is entirely separate from the area on the head, which is in turn separate from the radial process. In the smaller specimen the areas are all united. This is evidently a variation due to age, as the same thing is observable in large and small specimens of *Chelonia mydas*. The radial process lies near the center of the shaft, and is very prominent. It is simple, instead of having the U or V shape of the same process in existing sea turtles and in *Psephophorus*. The ento- and ecto-condyles and the entepicondylar and ectepicondylar processes are strong and prominent. The ectepicondylar foramen is quite large. The shaft of the bone was somewhat flattened and constricted beneath the head (Pl. VI, Fig. 12).

#### MEASUREMENTS.

Length from distal end to top of head . . . . .	.348 meters
Greatest width at distal end . . . . .	.165 "
Length from exterior edge of head to end of mesial process . . . . .	.175 "

The humerus shows a somewhat close resemblance to that of *Psephophorus* and *Dermochelys*. The radial process is simple, stands higher on the shaft, and lacks the downward prolonga-

tion shown in those forms. The higher position of the radial process is a primitive character, and is well shown in *Lytoloma* (33) and in *Chelonia girundica* (34), as figured by Delfortrie.

The *radius* and *ulna* are apparently represented by the proximal ends of two bones that from their size could not have belonged to the posterior extremity. They are so crushed as to afford no distinctive characters.

The *scapulae* show a broad angle between the scapula proper and the proscapular process. Both parts are strongly compressed, but show on their ends large areas for cartilaginous attachment. The neck of the glenoid portion is short in comparison with existing members of the *Cheloniidae*. The proscapular process is much the shorter. The glenoid articular portion shows two faces: one for the coracoid and the other forming part of the glenoid cavity. The whole bone is very strongly built (Pl. VI, Fig. 13).

The *coracoids* are long, slender bones, greatly thickened proximally where they articulate with the scapulae. Distally the shaft becomes flattened and very thin. Upon the upper surface there is a strong ridge running from the proximal end out into the flattened part of the shaft, where it disappears. This ridge is present in both *Chelonia* and *Dermochelys*, but is absent in *Thalassochelys*. In the latter form the whole bone is proportionately shorter and stouter (Pl. VI, Fig. 14).

#### MEASUREMENTS.

Length of most nearly complete bone . . .	.405 meters
Breadth distal end . . . . .	.075 "

The *pubis* has a very large and distally expanded ectopubis. It is much larger than the entopubis, and joins it at almost a right angle; in these respects it differs from the living sea turtles, where the two processes meet at an angle. The greatest axis of the ectopubis is in almost a line with the axis of the whole pubis. In the *Pinnata* these two meet at an angle. The entopubis joins the main body of the bone at almost a right angle by a very short and very broad neck, the anterior edge of which nearly reaches the edge of the acetabular face. The symphyseal faces of the entoschia were nearly straight,

so that they touched for a great part of their length. The process bearing the articular faces for the other bones of the pelvis is short and strong (Pl. VI, Fig. 15).

## MEASUREMENTS.

Length from proximal end to end of ectopubis . . . . .	.243 meters
" " external edge of bone to ischial symphysis . . . .	.169 "
" " point on shaft opposite center of entoschium to proximal end . . . . .	.057 "

The *ischium* is somewhat hourglass-shaped in profile, with the distal end the largest and the middle of the bone much contracted. The broader portion of the shaft is thin, and the anterior edge rounded and thickened. The symphyseal edge is somewhat convex, the two bones meeting probably in the middle portion only. The pubo-ischiatic foramen was small in *Dermochelys* (Pl. VI, Fig. 16).

The *ilium* is a short, strong bone, concave on its lower surface, and angularly convex above from before backwards. The distal articular surface is confined to the end of the bone. The center of the upper side is rugose for cartilaginous attachment (Pl. VI, Fig. 17). The figure of the ilium is made from the smaller specimen, as it is much more perfect than the larger.

The *femur* is much smaller and more slender than the humerus. The distal end is expanded. The shaft is contracted below the head, which was supported on a well-developed neck (Pl. VI, Fig. 18).

## MEASUREMENTS.

Length of femur . . . . .	.295 meters
Greatest breadth distal end . . . . .	.112 "
Breadth center of shaft . . . . .	.091 "

*Protostega* has, then, the following points in common with the *Cheloniidae*: the peripherals, the condition of the plastron (part.), the lack of such a large amount of intercalated cartilage in the articulations of the bones of the skull, the nature of the semicircular canals in the paroccipital, petrosal, and exoccipital, and the shape of these bones; the formation of the foramen for the external carotid by the petrosal and quadrate to the almost complete exclusion of the paroccipital, the form and position of



the quadrate, the form of the squamosal and its close articulation with the quadrate, the articulation of the paroccipital with the squamosal, the well-ossified and separated condylar portions of the exoccipital and basioccipital, the manner of articulation of the palatines with the maxillaries, the posterior nares (part.), and the form of the mandible.

With *Dermochelys* it agrees in, the broad basioccipital with its lateral processes preventing the articulation of the pterygoid and exoccipital, the broad basisphenoid separating the pterygoids on the base of the skull (to a less extent than in *Dermochelys*), the nonappearance of the pterygoids on the posterior aspect of the skull and their not being perforated by a branch of the carotid artery, the large groove on the pterygoid for the epipterygoid process of the quadrate, the large epipterygoid process of the quadrate, the posterior nares (part.), and the vomer, the lack of a carapace, the large nuchal, the humerus (part.), and the plastron (part.). There should also be mentioned here the stapes, which is even larger than in *Dermochelys*.

Points separating *Protostega* from both forms are the lack of dermal ossifications on the back, the manner of articulation of the pterygoid and quadrate, the presence of a presplenial bone in the jaw, the lack of any articular process on the under side of the nuchal, the simple radial process of the humerus, and the peculiar bent form of the xiphiplastr.

*Protostega* is distinctly an intermediate form.

In the paper containing the description of *Protostega* (2) Cope attempted a restoration from the material at his command. He estimated the head as  $24\frac{5}{8}$  inches long, and by assuming the proportions to be near those of *Chelonia*, the neck and carapace as  $138\frac{2}{8}$  inches, making a total length of  $154\frac{7}{8}$  inches, or 12.83 feet. (He evidently deducted 8 inches from the neck as remaining within the carapace.) From the ribs and vertebrae he estimated the width of the carapace to be  $36\frac{1}{2}$  inches, and the length 118 inches. The series of marginals did not justify this length, but he considered that they were not united, and that the intervening spaces would make up the deficiency. His final conclusion was that the carapace was more elongate and narrower than in any existing form of sea turtle.

In a recent paper (43), Dr. O. P. Hay has described portions of the plastron of a large specimen of *Protostega*, and attempted a restoration. The materials on which his restoration was based were the almost complete hyo- and hypo-plastra of one side and a fragmentary nuchal. Regarding the plastron he says (p. 58): "In *Thalassochelys* the anterior end of the epi-plastra extends in front of a line joining the bottoms of the excavations for the fore limbs a distance equal to that from the bottom of the excavations for the fore limbs to those of the hind limbs. This, in the *Protostega* plastron before me, amounts to 84 cm. The xiphiplastra of *Thalassochelys* extends behind the excavations for the hinder limbs as far as do the epiplastra from the anterior excavations. If these proportions hold good for *Protostega*, the whole length of the plastron would amount to at least 2.4 meters"; and further (p. 59): "had the breadth of the body of *Protostega* possessed the same ratio to the length that we find in *Thalassochelys*, the lower side of the animal would have been about 2.2 meters wide." In regard to the fontanelle: "if we have placed the plastral bones aright, there is left between them a great fontanelle. Where the hypoplastra are widest, this is about 43 cm. in width, and opposite the union of the hyo- and hypo-plastron about 90 cm. This is somewhat smaller, however, than the fontanelle found in *Protosphargis*, and much smaller than that of *Dermochelys*." The head he estimates as 32 cm., from the snout to the end of the occipital condyle, and concludes as follows (p. 62): "The length of the carapace of *Chelonia* has a ratio to the plastron of about 31 to 24. Hence the length of the carapace of my specimen must have been close to 3.1 meters. The neck of our living marine turtles projects beyond the front of the carapace a distance equal to at least  $\frac{1}{6}$  of the length of the carapace. Hence we are safe in allowing 50 cm. for the neck outside of the shell. We have, therefore, for the length of this turtle the following figures:

Head . . . . .	.32	meters
Neck beyond carapace . . .	.50	"
Carapace . . . . .	3.10	"
Total . . . . .	3.92	" "

The specimens just described afford material for quite accurate measurements, which give results different from those obtained by Cope and Hay, the main discrepancy being in the relative length of the carapace to its breadth. The present specimen shows the peculiar bent condition of the xiphiplastron, which was not indicated in the specimens described by the authors mentioned. This would account for a considerable reduction of the length of the plastron, and a still further reduction is quite certainly to be found in the condition of the epiplastron. In none of the known specimens has any trace of epiplastron been discovered, and neither in the specimen here described nor in Dr. Hay's specimen can I find any trace of attachment of the epiplastron. Moreover, the anterior ends of the hyoplastra meet over the anterior end of the fontanelle. In the plate of *Protosphargis* given by Capellini the restored epiplastron extend beyond the exterior end of the hyoplastra a distance of one-tenth the length of the plastron as restored. This restoration is open to doubt, however, as the close resemblance of *Protosphargis* to *Protostega* makes it possible that the distal ends of the xiphiplastron were incurved as in *Protostega*. Only the proximal ends of both epiplastron and xiphiplastron are known. It may be assumed, however, for the purposes of this restoration, that the epiplastron extended in front of the hyoplastra a distance of one-tenth the length of the plastron.

The distance from the posterior edge of the conjoined xiphiplastron to the anterior extent of the hyoplastra is 1.15 meters; adding to this one-tenth the length of the plastron, we have 1.27 meters, instead of 2.4 meters, as estimated by Hay.

Fortunately, in the process of fossilization, the nuchal plate was pressed down upon the plates below, preserving them in their normal position, and rendering it possible to give exact measurements of the fontanelle. It was bridged in its anterior and posterior extent by the processes from the plastral plates, and at its widest part measured .525 meters, instead of .90, as estimated by Hay.

If we assume the ratio of the carapace to the plastron as 31 to 24, as in *Chelonia*, the carapace was 1.64 meters long. In a three-fourths grown specimen of *Chelonia* the ratio of the

breadth of the plastron to the breadth of the lower surface of the turtle is as 5 to 6. The distance across the plastron in this specimen of *Protostega* in its widest place is 1.029 meters; and this, according to the ratio stated, would make the lower surface of the turtle 1.235 meters wide. The widest part of the carapace in *Chelonia* does not correspond with the widest part of the plastron, but is broader somewhat behind it, so the general form of the carapace was not long and narrow, but almost round.

As shown in Pl. V, Fig. 6, the quadrate, pterygoid, and palatine of the smaller specimen are all united and very slightly distorted by pressure, especially in a linear direction. The measurements of these bones, including length of quadrate, length of condylar face of quadrate, and length from posterior end of quadrate to anterior end of palatine, are almost exactly the same as that of a skull of *Chelonia mydas*, which measures .197 meters from snout to occipital condyle. The humerus of the smaller specimen is six-elevenths as large as the same bone in the larger specimen, both being in excellent condition. If it is assumed that the same ratio applies to the head, the larger specimen would have a skull measuring .363 meters from snout to occipital condyle.

No material is at hand to give exact measurements of the neck, but assuming with Hay that the neck extended in front of the carapace a distance equal to one-sixth of the carapace, it would have a length of .278 meters.

The exact figures are:

Plastron, from xiphiplastra to anterior end, hyoplastra . . . . .	1.15 meters
Breadth of fontanelle at suture between hyo- and hypo-plastra . . . . .	.525 "
Breadth of plastron at widest part of hyoplastra . . . . .	1.029 "

The estimated figures are:

Length of carapace . . . . .	1.640 meters
Length of head . . . . .	.363 "
Length of neck . . . . .	.270 "
Total . . . . .	2.273 "
Width of carapace . . . . .	1.235 "

In both Cope's and Hay's specimens the animal was a very little larger than in the present one.



*Systematic Relationship of Protostega and Allied Forms.*

The forms most important in this connection are:

*Dermochelyidae.*

*Dermochelys* Blv. (35).

*Psephophorus* v. Meyer (36).

*Eosphargis* Lydekker (30).

*Protostegidae* (3).

*Protostega* Cope (2).

*Protosphargis* Cap. (31).

(?) *Pseudosphargis* Dames (28).

*Cheloniidae.*

*Osteopygis* Cope (37).

*Allopleuron* Baur (20).

*Lytoloma* Cope (38).

And also the living forms of the *Cheloniidae*.

The known material is deficient in comparable portions, thus only a part of a head and nothing of the body is known of *Pseudosphargis*, while the skull is absent and the body very perfectly preserved in *Protosphargis*. *Eosphargis* is known from the skull and very incomplete body skeleton, and so on. Conclusions drawn from such material must be, in a sense provisional and await the evidence of future discoveries for confirmation.

The *Protostegidae* are characterized as a distinct group by the presence of descending parietal plates and the absence of a carapace. In the middle cretaceous form, *Protostega*, the descending parietal plates are well developed. In the upper cretaceous form, *Protosphargis*, as already stated, the skull is unknown, but the almost generic identity of the body skeleton with *Protostega* makes the presence of the plates very probable. In *Pseudosphargis* of the oligocene they are present, but the lower end has only a weak connection with the pterygoid; of this form, Dames says (28), p. 17, "Bei *Pseudosphargis* endlich bilden sie im oberen Theil noch wohlentwickelte Lamellen, die jedoch mit ihrem Vorderrande weit hinter der erwähnten Verbindungslinie der Orbitae zurückbleiben, im untern Theile sind sie auf schwache Pfeiler reducirt, deren gänzliches Verschwinden eine sphargis-

ähnliche Ausbildung ergeben würde." It is placed among the *Protostegidae* upon the evidence of the descending processes; but, as will be shown later, the flat, wide skull has a strong resemblance to some of the *Dermochelyidae*.

No forms of this family have shown the presence of dermal ossifications in the carapace, but all, in which the carapace is known, do have peripherals which are unknown by observation in the *Dermochelyidae*.

The nuchal plate of the middle cretaceous form, *Osteopygis*, is known to lack the process on the under side for the last cervical vertebra. The other forms, probably, were devoid of the process.

The *Dermochelyidae* have a carapace formed of dermal ossifications, no peripherals, and an entire lack of the descending plates of the parietals. The earliest known form, *Eosphargis*, from the eocene, has the carapace represented by a median row of scutes which are, possibly, the loosened and expanded neural plates; the peripherals were supposed to have been present by Lydekker (30). The skull shows no trace of the parietal plates, and is broad and flat.

The next form, *Psephophorus*, ranges from the eocene into the miocene. It has the tessellated dermal ossifications of the carapace already well developed, no peripherals, and the humerus very similar to that of *Dermochelys*.

The *Cheloniidae* afford better material for comparison. The earliest form, *Osteopygis*, from the middle cretaceous, had eleven peripherals, 2, and 11 were free from rib attachment, 2 and 8 had deep pits for attachment to the plastron. Between these there was a small fontanelle. The carapace was practically closed.

*Allopleuron* (*Chelone Hoffmani* Gray) of the upper cretaceous presents an evident offshoot from the true line of the *Cheloniidae*. The carapace was long and narrow, the nuchal deeply emarginate, and the neurals short and wide with a long keel. The pleurals are of considerable antero-posterior extent, but are confined to the proximal ends of the ribs, which are very slender. The nuchal shows no process on its under side. The peripherals are long and slender. The posterior nares are located far back.

*Lytoloma* of the upper cretaceous and lower eocene has eleven peripherals; 1, 2, and 10 are free, the third has a small pit for a process from the plastron. According to Baur (22), the specimens in the Bruxelles Museum have no pit. The nares and orbits are directed upward. The palatal aspects of the temporal fossae are wider than long. The ecto-ptyergoid process is near the anterior extremity of the pterygoid. The posterior nares are in the hinder half of the cranium. The vacuities of the shell are even less than in the recent *Thalassochelys*. The nuchal in the eocene form has the beginning of a process for the cervical.

*Argillochelys* from the eocene has the orbits and nares directed slightly upward. The palatal apertures of the temporal fossae are as wide as long. The ecto-ptyergoid process is at the antero-external angle. The posterior nares are in the anterior half of the cranium. The carapace was, probably, very close to that of *Thalassochelys*.

*Thalassochelys*, eocene and recent, has more than 11 peripherals, the addition taking place between the 1 and 3. 1, 2, and 9 are free from ribs; there are no pits for the plastron. The carapace is completely ossified in the adult. The posterior nares are in the middle half of the skull, retreating as age advances. The nuchal has a process for the cervical vertebrae.

Before attempting to interpret the facts just given, it may be of value to review briefly the discussion between Baur, Dollo, and Boulenger on the systematic position of *Dermochelys*, and the validity of the group *Athecae*.

Baur in 1886 (19) declared that the separation of *Dermochelys* from the rest of the *Testudines* was a purely artificial one: "Diese Absonderung der *Dermatochelyidae* ist keine natürliche sondern eine absolut künstliche," giving as his reasons:

1. That the configuration of the skull and of its separate elements is directly comparable to that of the *Cheloniidae*, and especially to *Eretmochelys*.

2. The cervical vertebrae are like those of the *Cheloniidae*, the fourth being biconvex.

3. The nature of the claws. In *Thalassochelys* the first and

second digits have claws; in *Eretmochelys* the first and sometimes the second; and in *Dermochelys* there are no claws.

4. The plastron of *Dermochelys* is reduced, not primitive. In the cretaceous *Protostega* and *Protosphargis* the plastron is much more strongly developed than in *Dermochelys*. These forms also lack the entoplastron, which is present in the *Cheloniidae*. The remaining elements of the plastron are directly referable to that of the *Cheloniidae*, though the hyo- and hypoplastron are not united by suture.

5. In addition to the mosaic-like carapace of *Dermochelys* there is present a nuchal plate which is comparable to that of the *Cheloniidae* and to it alone.

His conclusion was that the only difference between the *Athecae* and *Thecophora* is in the form of the carapace and its complete separation from the inner skeleton, that the ancestors of the *Athecae* had the carapace united with the inner skeleton, and that the peculiar carapace was formed by the breaking up into small pieces of the original carapace; giving as evidence of this process the case of a specimen of *Eretmochelys*, in which he observed that the costal plates from the third to the sixth had separate ossicles of bone on their edges. He also cited the reduction of the peripherals of *Eretmochelys* and their complete disappearance in *Dermochelys*, and the peculiar anterior rib in both forms.

To this paper Dollo (9 and 10) replied in detail, maintaining the natural value of the group *Athecae*.

1. He considered that if *Dermochelys* was descended from the *Cheloniidae*, in which there are always fontanelles in the carapace, that there should be some in the carapace of *Dermochelys*. He regarded a supposition that such fontanelles existed, but had been filled up by the dermal ossifications as improbable. He says (9), p. 165, "Or préfère-t-on supposer que les ancêtres des Chélonées avaient une carapace sans fontanelles et que, de cette souche, se seraient développés par delamination, les Athèques, et, par formation de fontanelles, les Chélonées actuelles?"; however, he considered this as improbable, as both the embryological evidence of living forms and the paleontological evidence of the earliest known turtle (*Thalassemy*



Rütimeyer) is in favor of the presence of fontanelles in the carapace.

He found difficulty in explaining, on Dr. Baur's hypothesis, how the plastral bones could still be present in *Dermochelys*, and separate from the external layer of dermal ossifications. He considered the dermal ossifications of *Ostracion*, *Polacanthus*, and *Glyptodon* to be of the same origin as those in *Dermochelys* and that no one would imagine that they came from the ribs.

He saw no relation between the bones of the skull in the two families. The shape of the pterygoids and the posterior nares located far forward militated against the idea of aquatic adaptation.

He showed that the series formed by Baur on the possession of claws was inaccurate, that *Eretmochelys* had two claws, and that the genus was formed for the reception of forms with two claws.

He regarded the plastron of *Dermochelys* as likely to be as much primitive as reduced.

The nuchal plate might be a more ancient form than the other peripherals, and have its origin in the necessity for a strong attachment for the neck muscles.

The articular faces of the cervical vertebrae he considered to be too variable to afford safe evidence of affinity.

He questioned the presence of peripherals in *Protostega*, and supposed that the peculiarity of the anterior rib was not necessarily related to the possession of the carapace, but might have been derived from ancestral forms.

In the same year as Dollo's latest paper appeared one by Boulenger (13), in which he said: "For my part I have to say that the statement that *Dermochelys* differs from the *Cheloniidae* only in the configuration and isolation of the carapace is simply monstrous. . . .

"He [Dr. Baur] actually states the head and limbs are fundamentally the same in *Dermochelys* and in the *Cheloniidae*. The skull of the former bears a general resemblance to that of the true turtles; but this is limited to the shape, and, to a certain extent, the general constitution of the temporal roof; in the absence of the column-like processes of the parietals, descend-

ing to the pterygoids in front of the supraoccipital and the prootics, it differs from that of all other *Chelonians*. Thus, in addition to the shape of the humerus and the proportions of the phalanges, the fore limb differs in the radius and ulna being subequal in length and placed side by side in a horizontal plane, and in the fifth metacarpal, instead of the first, being the shortest."

In 1889 appeared Baur's reply to these papers (23). Taking up Dollo's objections first, he stated the belief that the fontanelles in the carapace of *Dermochelys* might be filled up by the expansion of the elements of the dermal carapace, after they had lost connection with the inner skeleton, citing as an example of such disappearance of the fontanelles the case of an old specimen of *Aspidonectes (Anyda) muticus*.

He showed that the oldest known specimen of turtles was not *Thalassemys* Rütimeyer, with fontanelles, but *Proganochelys Quenstedtii* Baur, which had no fontanelles; also, that in some of the living sea turtles the carapace becomes closed in old age (*Colpochelys* and *Thalassochelys*). He showed that the direct ancestors of the sea turtles had no fontanelles in the carapace, and that the plastron was stronger than in recent forms. To the objection that the embryonic forms showed fontanelles in the carapace he replied that the principles of embryology could not be used in interpreting the meaning of the ontogenetic development of dermal ossifications.

He applied the statement that the ontogenetic development of the exoskeleton was of slight morphological value to the statement by Dollo that if Baur's hypothesis was a true one, the embryo should show simple ribs becoming confluent, and again single, and also to the objection that the dermal ossifications overlay the plastral elements from which they were supposed to take origin.

He showed that the posterior nares were the same in *Dermochelys* as in the *Cheloniidae*, that the nature of the articular faces of the cervical vertebrae was constant, and that the plastron of *Dermochelys* was reduced from a stronger form. He reestablished his series based on the claws as follows: "Bei *Caretta (Eretmochelys)* sind gewöhnlich 2 Klauen vorhanden

ebenso bei *Thalassochelys*, dass aber bei *Chelonia*, *Lepidochelys*, und *Colpochelys* im allgemeinen nur eine Klaue vorkommt. *Dermochelys* besitzt gar keine."

He showed that the presence of peripherals in *Protostega* and *Protosphargis* was no longer to be questioned.

In reply to Boulenger's paper he brought forward additional evidence of the relationship between *Dermochelys* and the *Cheloniidae*. He concludes (p. 190): "Ich habe früher [*Science*, 1888] die Vermutung ausgesprochen dass *Dermochelys* von einer Form der *Pinnata* mit vollkommenem Rücken- und Bauchschild abstamme, während ich annahm, dass *Protostega* und *Protosphargis* von den *Cheloniidae* durch Vermittlung von *Allopleuron* (*Chelonia Hoffmani*) sich entwickelt haben. Es scheint mir jedoch wahrscheinlicher, dass *Dermochelys* und *Psephophorus* direkt auf *Protostega*- oder *Protosphargis*-ähnliche Formen zurückführbar sind, und dass der mosaikartige Panzer möglicherweise eine Neubildung darstellt." Again: "Darüber aber ist kein Zweifel, dass *Dermochelys* und *Psephophorus* keine ursprünglichen Formen sind, sondern dass sie von wahren 'Thecophoren' und zwar von den 'Pinnaten' abstammen, um mich hier dieses Ausdrucks zu bedienen."

Dr. Baur has since expressed his belief that the carapace of *Dermochelys* was not formed, as he formerly supposed, by the breaking up of the plates of the original carapace, but by a secondary ossification of the integument after the neurals and costals had disappeared, the state observed in *Protostega* and *Protosphargis*.

The matter stood thus unsettled before the present specimens were known. They clearly show *Protostega* to be an intermediate form; but was it a step in a series advancing from *Dermochelys* to the *Cheloniidae*, or a specialization of the primitive *Cheloniidae* toward *Dermochelys*? I believe the latter.

There can no longer remain a doubt as to the possibility of referring the skull of *Dermochelys* to that of the *Cheloniidae*; *Protostega* fills the gap completely. The lost neurals and costals of *Dermochelys* are seen in the expanded ribs of *Protostega*, and the vanished peripherals are strong in the cretaceous genus. The lack of a process on the nuchal of *Protostega* speaks strongly for the origin of *Dermochelys* by specialization

from the stem of the *Cheloniidae*. Is it likely that the primitive form would have this process, lose it in the cretaceous, and regain it in the upper eocene and later forms? It is more likely that the *Dermochelyidae* arose after the process had appeared in the main stem, and the *Protostegidae* before it. Nor is it likely that the process is secondary in either of the families possessing it. In the cases where such parts are seemingly reproduced in nature, it is generally found that some neighboring part has assumed the function and appearance of the lost part; there is nothing here that could have done this.

*Allopleuron* connects *Protostega* with the main stem of the *Cheloniidae*; it cannot be in the direct line, as it is upper cretaceous, while *Protostega* is middle cretaceous; but its immediate ancestor must have been the connecting form. As shown in the description given above, and as will be more readily seen by reference to the plates in M. Ubagh's two papers (41, 42), the skull is closely related to that of *Thalassochelys*, while the slender ribs and reduced pleurals are a direct step toward the state found in *Protostega* (see Pls. I, VII, XII, and XIII of Winkler's monograph, 40).

The point of approach of *Protostega* to the stem is indicated by the lacking process on the nuchal. In *Osteopygis*, the cretaceous form, the process is wanting, while in *Lytoloma* of the upper cretaceous and in the form of the lower eocene it is present. Evidently *Protostega* branched off before the time of *Lytoloma*.

*Protosphargis* of the upper cretaceous is the next step in the *Protostegidae*, as evidenced by its appearance in time, its simpler ribs, and reduced peripherals and plastron.

*Pseudosphargis* is placed as the last of the known *Protostegidae* because of the presence of the descending parietal plates and its appearance in the oligocene. The lack of any portion of the animal beyond the posterior part of the skull makes the determination of its systematic position doubtful. If it should prove to have a process on the nuchal plate for the eight cervical vertebrae and any trace of a dermal carapace, it might, though occurring so late, be regarded as representing the first step toward *Eosphargis*. This view would be supported by the weak attachment of the lower end of the parietal plates.

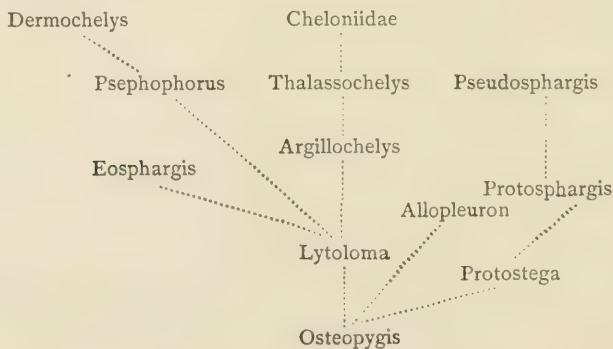


In a slightly different direction developed those forms with the parietal plates absent, and dermal ossifications in the carapace and plastron culminating in *Dermochelys*. The first form to appear was *Eosphargis*, slightly off the main line of the *Dermochelys* branch. The primitive form with parietal plates and lacking dermal ossifications has yet to be found to connect the branch with the main stem. From the strong resemblance of *Eosphargis* to *Dermochelys* it is fair to say that it had a process on the nuchal plate, and if so it must have come from a form which left the main stem after *Lytoloma* had developed the nuchal process. That it is slightly different from the rest of the branch is shown by the peculiar dermal carapace, consisting of the large carinated dermal scutes, instead of the small ones of *Psephophorus* and *Dermochelys*. It may be that these scutes are the last of the neurals; if so it represents a stage in its branch corresponding to a stage between *Allopleuron* and *Protostega* in the other branch.

The second member of this branch is *Psephophorus*, running from the eocene to the pliocene; it has been shown by Dollo to be unquestionably a member of the *Dermochelyidae*, with well-developed dermal ossifications in the carapace, complete loss of neurals and peripherals, and a characteristic humerus.

*Dermochelys* is the culminating form of this branch. The series *Osteopygis*, *Lytoloma*, *Argillochelys*, *Thalassochelys*, representing stages in the main line of the *Cheloniidae*, needs no further explanation than the facts set forth above.

The ideas here expressed may best be seen by reference to the following diagram:



We may then conclude that *Protostega* is a connecting link between *Dermochelys* and the *Cheloniidae*. The evidence for this position of *Dermochelys* may be stated as follows:

(1) The bones of the head are referable to those in the head of the *Cheloniidae*. The intermediate form is represented in *Protostega*.

(2) The cervical vertebrae are alike in both families. The fourth is biconvex and the articular surfaces between the sixth and seventh are flat.

(3) The plastron of *Dermochelys* is a reduced form of the plastron of the early *Cheloniidae*. The intermediate stages are represented in *Protostega* and *Protosphargis*.

(4) The carapace is composed of dermal ossifications; they appeared after the bony carapace had disappeared by ossification of the integument. The original carapace was removed by the enlargement of its lateral fontanelles. An intermediate form, with the carapace gone and the separate ossifications not yet formed, is represented in *Protostega* and *Protosphargis*.

(5) The process of the disappearance of the peripherals is known in all stages.

(6) The nuchal plate of *Dermochelys* is provided with a process for articulation with the last cervical; this process is absent in *Protostega* and present in the *Cheloniidae*. The plan of development could not have included, in the time occupied, its loss and subsequent reacquisition. The line of *Dermochelys* rather took origin after the process was developed in the main stem of the *Cheloniidae*.

In conclusion I wish to express my thanks to Dr. G. Baur and to Dr. S. W. Williston for material most kindly furnished me for these studies.

PALEONTOLOGICAL LABORATORY, UNIVERSITY OF CHICAGO,  
April, 1896.

#### ADDITIONAL NOTE.

Since the above was written Mr. G. R. Wieland has described the remains of a large sea turtle from the upper cretaceous of

South Dakota.<sup>1</sup> The description includes the carapace, vertebrae, limb bones, and pectoral girdle, which he regards as indicating a new genus and species, *Archelon ischyros*. Nothing is added to our knowledge of the morphology of the extinct sea turtles, except the presence of a row of neural plates. This point shows my conclusions as to their absence to have been an error.

Mr. Wieland bases his new genus largely on the disparity in size between it and Cope's specimens of *Protostega*, and on several minor differences in the vertebrae, scapulae, and coracoids. The difference in size between two forms can rarely be used as a criterion for determining their generic individuality. For one accustomed to the great range of this feature in the fossil reptilia, and the persistent, though slow, growth throughout life of many recent forms, the use of this character seems attended with grave danger. The present paper was founded on two specimens of *Protostega*, one about half the size of the other, while Cope's described specimen is intermediate in size. The "minor development of the smaller trochanter" attributed to *Archelon* (p. 406), and the presence of "longitudinal depressions" on the shaft of the proscapular process of the scapula (procoracoscapular), instead of a "rotund" outer edge (p. 404), are, with characters of a like nature, features which might be readily produced or destroyed by the compression from which all specimens from the Kansas chalk suffer.

Mr. Wieland further speaks of the greater breadth of the carapace of *Archelon* as compared with *Protostega*. His conclusions are based on the calculations of Cope and Hay which I have shown above to be erroneous. His specimen has a length of 3.52 meters, and a breadth of 2.25, a little less than two-thirds, while mine is a little over one-half as wide as long. *Archelon* must be considered as a synonym of *Protostega*, and even its specific separation remain an open question.

December 14, 1896.

<sup>1</sup> *Archelon ischyros*: a New Gigantic Cryptodire Testudinate from the Fort Pierre Cretaceous of South Dakota. Am. Jour. Sci., vol. ii, December, 1896, p. 399, 1 Pl.

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## EXPLANATION OF PLATE IV.

Photograph of plastron with nuchal plate and peripherals.















## EXPLANATION OF PLATE V.

FIG. 1. Supraoccipital  $\frac{1}{2}$ . The badly crushed petrosal and paroccipital are not shown.

FIG. 2. Exoccipital  $\frac{1}{2}$ . Right side.

FIG. 3. Basioccipital  $\frac{1}{2}$ . *a*, from above; *b*, from below.

FIG. 4. Petrosal  $\frac{1}{2}$ . *a*, from within; *b*, from without.

FIG. 5. Paroccipital  $\frac{1}{2}$ . Right side.

FIG. 6. Quadrate, pterygoid, and palatine  $\frac{1}{2}$ . Right side.

FIG. 7. Quadrate-jugal  $\frac{1}{2}$ . Right side.

FIG. 8. Lower jaw.

FIG. 9. Xiphiplastron  $\frac{1}{3}$ . Left side, showing attachment with hypoplastron and xiphiplastron of right side.

FIG. 10. Nuchal plate a little over  $\frac{1}{3}$ .

FIG. 11. Fifth and sixth peripherals. Left side.













## EXPLANATION OF PLATE VI.

- FIG. 12. Humerus  $\frac{1}{3}$ . Right side.  
FIG. 13. Scapula  $\frac{1}{3}$ . Right side.  
FIG. 14. Coracoid  $\frac{1}{3}$ . Right side.  
FIG. 15. Pubis  $\frac{1}{3}$ . Right side.  
FIG. 16. Ischium  $\frac{1}{4}$ . Right side.  
FIG. 17. Ilium  $\frac{1}{4}$ . Right side (taken from smaller specimen).  
FIG. 18. Femur  $\frac{1}{3}$ . Left side.  
FIG. 19. Rib head  $\frac{1}{2}$ . (Taken from smaller specimen.)







12.



11.



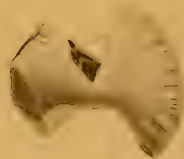
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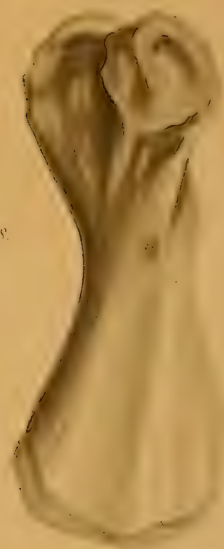
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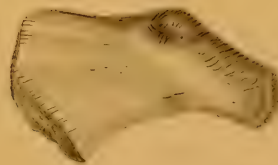
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15.



17.





# THE INNERVATION OF THE AUDITORY EPI- THELIUM OF MUSTELUS CANIS, DE KAY.<sup>1</sup>

ALBRO D. MORRILL.

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## I. *Historical.*

1. *Recent work on auditory epithelium.* — The application of the methods of Ehrlich and Golgi in their original and variously modified forms to the study of the auditory epithelium has greatly increased our definite knowledge of its innervation.

Although there is considerable general uniformity in the results obtained by different observers, there are several disputed points, particularly in the interpretation of the results obtained. One of these which is of the greatest importance is the exact relation of the terminal branches of the nerve fibers to the auditory or hair cells.

The numerous investigations carried out by Retzius by the old methods, as well as by the most recent, have led me to give a brief statement of the results of his earlier observations before considering more recent conclusions.

In his great work, Retzius ('84) states that in general he was able to trace the nerves into the auditory epithelium and follow the naked nerve fibers between the basal layers of the support-

<sup>1</sup> I wish to express my indebtedness to Dr. Howard Ayers, at whose suggestion the work was undertaken; also to the Director of the Marine Biological Laboratory at Woods Holl, Mass., for many privileges enjoyed during the progress of the work.



ing cells in a nearly perpendicular direction until about half the height of the epithelium was reached. At this point branches were given off extending horizontally at the base of the hair cells, and each was applied quite firmly to from three to five hair cells at their proximal ends. The determination of the exact relation existing between the nerve fibers and the hair cells was not accomplished, owing to the inadequate methods then in use.

In the ear of man he describes a shell-like expansion of the nerve at the base of the hair cell in which these cells were supported. In the pigeon's ear he saw fine varicose nerve fibers ascending between the hair cells, as had been already observed by several investigators.

That the relation of the nerve fibers to the hair cells was uncertain is shown in Retzius' account of the auditory epithelium of the rabbit: "Ihre definitive Endigung an oder in den Haarzellen ist deshalb gewissermassen noch ein ungelöste Frage." Again, in the account of the cat's ear: "Es scheint also als ob die feinen Fibrillen sich dem Protoplasma Haarzellen anlegten und sich an demselbern einigermaßen befestigten ob sie aber in dasselbe eindringen ist sehr schwer zu entscheiden."

Kaiser ('91), with the Golgi method, found the nerves of the auditory epithelium ending in cup-like structures, which were hyaline and contained numerous highly refractive granules. The hair cells fit into these structures, as he says, like eggs in egg cups.

Kaiser considers these cup-like structures as made up of nervous material, which form a connection between the nerves and hair cells. On this account he considers them of the greatest importance.

Ayers ('92) states that in the cochlea the sensory or hair cells are directly continuous with the fibers of the cochlear nerve. In a paper ('93) devoted wholly to a study of the relations of the auditory nerve and hair cells the previous statement is confirmed. "From the center of the base of each hair cell issues a nerve fiber which in a favorable case admits of being traced through a ganglion cell of the cochlear ganglion into the collection of fibers which pass to the brain." These fibers were

found to exhibit many varicosities in their course which vary greatly in size. The largest varicosities were nearly as large as the ganglion cells. It was thought, from results still unpublished obtained with Ehrlich's method, that these varicosities were of a cellular nature, probably sheath cells. The nerve fibers stained in this way were found to be uniform in size.

Dr. Ayers considers the hair cell as a genuine nerve cell and the cell of origin of the auditory nerve fiber. He thinks that at an early stage of development the ganglion cells are produced by the division of the superficial hair cells, and as development advances a protoplasmic thread connects the two cells. The nerve fiber soon begins to develop from the proximal end of the bipolar ganglion cell and extends to the brain. The relations existing between the hair cells and nerve fibers in the maculae and cristae acusticae were found to be essentially the same as in the cochlea only simpler. In the summary we find these statements:

"That there is no fundamental difference between the acoustic and olfactory elements yet made known."

"That all fibers of the auditory nerve proceed out of hair cells alone so far as has yet been satisfactorily determined."

Niemack ('92), in his observations on the auditory epithelium of the frog and rabbit with Ehrlich's method, found that the nerve after losing its sheath divided dichotomously many times. The final branches were of equal size, and, after rising to the level of the proximal ends of the hair cells, extended horizontally, forming a close network about their bases or a kind of sieve, in the meshes of which the hair cells rest. He found, as Kuhn had previously done in fish and amphibia, two kinds of nerve endings: one connected with the proximal ends of the hair cells, while in the other the nerve fibers pass in the interstices between the hair cells to end free at the surface in knob-like enlargements. Niemack found triangular swellings in the nerve fibers at their point of branching, as had been previously observed by Retzius. In the maculae he found a granular layer separating the nerve fibers from the base of the hair cells; a thin mantle of violet granules was also found covering the surface of these cells.

When examined with high powers, the dark varicosities of the nerves could be distinguished. He concludes after careful examination that the granular mantle had no connection with the nerve fibers: "Mit Nervenfasern zeigten sie keinen zusammenhang."

Geberg ('92) found both kinds of nerve endings: one free in knob-like enlargements at the surface between the hair cells, and the other in nerve fibers attached to the surface of the cells, but not continuous with them.

Retzius ('92) did not obtain satisfactory results with Ehrlich's method, but with Cajal's modification of Golgi's method used on embryonic or very young chickens and mice he secured good results. In comparing auditory hair cells and olfactory nerve cells, he says: "Die Haarzellen sind deshalb keine Nervenzellen sie sind den Riechzellen keineswegs gleichzustellen."

The bipolar ganglion cells of the acusticus are considered by him to be the true auditory nerve cells and correspond with the olfactory nerve cells. In numerous preparations he never saw a nerve fiber arising from a hair cell. The hair cells he classes as secondary sensory cells.

Retzius, who had previously described a structure similar to the hyaline cup observed by Kaiser connecting the nerves and hair cells, does not accept the latter's view in regard to its nature. In 1893 Retzius confirmed his earlier observations by the study of rat and trout embryos. In this publication he states that he does not always find Kaiser's cup-like structure present.

In the rat the hair cells were seldom stained, and then were of a clear chestnut-brown color, which did not interfere with the study of the relation of the nerve fibers and hair cells.

Van Gehuchten's results, obtained wholly independently of Retzius, were almost exactly the same in the main points.

Lenhossék ('94) found in his study of the auditory epithelium of young mice that supporting cells were frequently stained a deep black with the Golgi method, while the hair cells when stained at all were of a clear brown color. As Retzius had previously observed, he saw the deep black nerve fibers clearly outlined on the surface of the hair cells. He did not find the

nerve fibers extending so near the surface as Retzius ('93), Kaiser ('91), Niemack ('92), and Geberg ('92), but thinks the difference between his results and those of Retzius may be due to the age of the embryos studied. At the point of division or separation of the nerve fibers he observed the characteristic triangular thickening, and says that the nerve fibers were of nearly equal size. He did not find the wide-meshed network of nerves described by Niemack as existing at the base of the auditory epithelium of the frog and rabbit. The horizontal nerve fibers at the base of the hair cells were generally toothed on their upper surface, mainly at points where branches arise. He did not find any free endings at the surface and questioned the value of Niemack's sections, in which fibers were traced to the surface. Nothing was observed to support Kaiser's view. The nerve fibers which did not end free terminated in thickened knobs in contact with the surface of the hair cells. No more than two or three branches were observed to end in contact with a single hair cell. The number of nerve fibers was so great that their relations could only be studied when isolated nerves and their branches were stained. Lenhossék calls attention to the fact that it is easy for an inexperienced observer to be deceived when many nerves are stained, and to be led to think that anastomoses exist. In thick sections the tangle of fine varicose horizontal fibers and their cut branches at the base of the hair cells may easily be taken for a granular mass. This is, he thinks, the granular substance described by Kaiser and Niemack. Three layers are described in the maculae and cristae acusticae: (1) a hair-cell zone in which there are two layers of crowded cells arranged perpendicularly to the surface; (2) the nerves forming a "plexiform stratum" at the base of the hair cells; (3) the supporting-cell zone with the cells placed vertically.

On the basis of the fact that in his preparations the nerves never extended to the surface, he concludes that the hair cells are the medium through which the movements of the endolymph are conducted to the nerve fibers. He does not think that there is any intermediate substance connecting the nerves and hair cells, but that the peripheral portion of the cell pos-



sesses different chemical or physical properties from its central portions, and that this will explain its physiological action.

Cajal ('94) agrees in fundamental points with Retzius, van Gehuchten, and Lenhossék. He found some of the nerves ending free not far from the surface in varicose enlargements, while other similar fibers terminate in a very small number of cases outside the limits of the *cristae acusticae*. The nerve fibers were varicose.

The branches distal to the bipolar cells he considers as protoplasmic processes, while the smaller nerve fibers extending internally from the bipolar cells are the true nerves.

He did not find the network of nerve fibers below the hair cells described by Lenhossék, but thinks it probably due to the fact that they did not study animals at the same stages. Cajal studied foetal rats, while Lenhossék made his observations on rats several days old.

Retzius ('94a) later studied successive stages in the reptiles, and arrives at the important conclusion that the sensory nerve fibers grow from within outward: "Weil es zeigt das Nervenfasern von centraler Seite nach Peripherie hin wachsen und nicht von Anfang an von den Haarzellen entspringen oder mit ihnen zusammenhängen." This statement, if confirmed, will explain Lenhossék's finding the free endings so far from the surface. He also found, as in his earlier observations, some of the nerves ending in contact with the hair cells, but a greater number passed between the hair cells to end free near the surface.

There was never a direct connection of nerve fiber and hair cells, although both may be blackened by the chromate of silver so that they appear to be connected. In a single instance he saw a nerve fiber extending into the general epithelium of the ampulla. Whether these fibers were sensory or not could not be determined. Branching of nerve fibers, after passing the proximal ends of the hair cells, was occasionally noticed.

Retzius ('94b) confirms Ayer's ('93) observation on multipolar cells in the cochlear ganglion in finding cells with three processes, but did not find those with more processes. He thinks that Lenhossék lays too much stress on the horizontal distribu-

tion of the nerve fibers at the base of the hair cells. He found the nerves branching at any point and does not think there is a "nervum plexiforme."

2. *Recent work on nerve endings in frog's tongue.*—The close similarity of the nerve endings found in the terminal discs of the frog's tongue and those in the auditory epithelium make it important that a brief statement should be made of the results obtained by recent investigation.

Fajersztajn ('89) gives the following account of the distribution of the nerves in the epithelium of the terminal disc of the frog's tongue. After forming the subepithelial plexus, the fine nerve fibrillae become varicose and pass between the proximal cells of the disc, and in some cases extend vertically to the surface, where they end in distinct enlargements (*boutonné*) among the distal extremities of the cylindrical cells.

The terminal enlargements do not generally differ in any way from the ordinary varicosities, but in a few cases are larger and present certain differences in structure.

Ehrlich's method, he says, renders it impossible to determine whether the terminal enlargements are found between the cells or rest upon the cell membrane; probably they adhere to the surface of the cells.

The fibrillae which pass into the epithelium give rise to ramifications, and the lateral branches terminate in enlargements.

The terminal enlargement, intensely colored, is surrounded by a transparent vesicle.

The arrangement of the nerves and their mode of branching, as found by Niemack ('92) in the terminal discs of the frog's tongue, are essentially the same as he found in the *crista acustica* of the same animal.

In the subepithelial plexus he found many anastomoses; the nerve endings were rare in this region. From this network fibrillae passed between the cells to end free at the surface in knob-like thickenings. A second kind of nerve ending is found associated with certain cells. The end of the nerve is enlarged and attached to the proximal end of the cell.

This attachment is destroyed by teasing the preparation, and the cell and nerve are separated.

The cells associated with the nerve endings he considers as nerve cells. He found contiguity but no continuity. Niemack considers it possible that the two kinds of ending indicate two different functions. In his figures the nerve cells are represented as deeply stained.

Bethe ('94), in his study of the frog's tongue and palate with a modification of Ehrlich's method, found two kinds of cells reaching the surface of the terminal disc and several kinds of nerve endings:

1. Free endings between the cells and reaching the surface.
2. Endings on or in the epithelial cells.
  - (a) On cylindrical cells with three lobes (dreilappigen).
  - (b) With round end plate on rod cells, forked cells, and deep cylindrical cells.

These different endings may come from branches of the same nerve.

The terminal enlargements were found by Bethe on the margins of the terminal discs and sensory elevations rather than in the center.

He questions the accuracy of Niemack's conclusion that a free ending is found between every two cells. The second kind of ending is found in the middle of the terminal disc and is generally enlarged and knob-like at the end.

The terminal enlargement of the nerve is firmly adherent to the cell, and admits of the cell being moved about without becoming detached. With a one-sixteenth oil immersion it appeared to be intimately joined to the cell.

No continuation of the terminal enlargement or end plate into the cell was observed.

The nucleus was counterstained with alum cochineal and could be easily distinguished, except in the cylindrical cells, where it was hidden by granular matter.

Bethe claims to be the first to demonstrate the threefold nature of the nerve endings. They show a distinct clover-leaf shape from the surface, but look like flat discs when seen from the side.

The position of the nerve ending on the cell varies, being at

different heights either above or opposite the nucleus. It was never found proximal to the nucleus in the cylindrical cells.

The nerves end in a single rounded end plate proximal to the nucleus in the rod cells.

The third kind of ending was found in connection with cells which do not reach the surface and in which the nucleus is placed at right angles to the long axis of the cell.

Bethe does not think that the blue coloration of the cell is any indication of its sensory nature, as he found quite a variety of cells stained in that way; the color was superficial.

He found nerves ending in connection with gland cells, ciliated epithelial cells, and in deep epithelium cells distinguished by dark nuclei.

On the ciliated cells the clover-leaf-shaped end plate was found in contact with the cell. Frequently a small branch of the nerve extends to the surface and ends free without a terminal enlargement.

The dark nucleated cell with the round end plate is sparsely scattered through the epithelium.

Bethe has seen varicosities form on the slide in living nerve tissue of the crayfish, and considers those seen in nerve preparations as artifacts.

## II. *Methods.*

In recent study of the auditory epithelium, embryonic material has been treated by the rapid Golgi method, and adult tissue with Ehrlich's method.

In my investigation of the auditory epithelium of adult *Mustelus*, I was unable to secure any results with Golgi's method, although many experiments were tried.

At first Ehrlich's method gave widely different results, but after considerable experimentation it was found that by using only active, healthy fish killed by decapitation as soon as removed from the water, it was possible to secure uniformly good results by using certain precautions.

The ampullae were removed immediately and placed in enough normal salt solution to cover them. A Minot solid



watch glass was used for this purpose. A  $\frac{1}{2}\%$  solution of methylen blue in normal salt was added in sufficient quantity to produce a deep blue color, but not enough to render it opaque. Very little of the blue was required. The staining fluid was occasionally agitated by forcing air through it by means of a medicine-dropper. The temperature was kept between 80° F. and 90° F. The best results were obtained on the hottest and driest days in August. A warm plate or thermostat was used to maintain the proper temperature when needed on cool days. At the end of an hour to an hour and a quarter the stain was removed by means of a pipette, and the specimens rinsed with normal salt solution to remove excess of stain. They were then exposed to the air for ten to fifteen minutes, care being taken to keep them moist with normal salt solution. At the end of this time the specimens were transferred to the fixing fluid, which consisted of a saturated solution of picrate of ammonia in distilled water, to which one-third of its volume of normal salt solution was added. Two or three drops of 1% osmic acid was also added to every 10 cc. of the fixing fluid. The osmic acid prevented maceration and blackened the medullated nerve fibers. After treatment with the fixing fluid for about an hour and a half, the ampullae were transferred to a saturated solution of loaf sugar in distilled water for one hour. The syrup was removed from the surface of the specimens with blotting paper, and they were placed in a saturated solution of pure gum arabic in water for fifteen minutes. The ampullae were then placed one at a time in a drop of the gum arabic solution on the plate of a freezing microtome, and after careful orientation were frozen. The freezing was accomplished by means of liquid carbonic acid. The apparatus<sup>1</sup> used was modeled after the one designed by Dr. Mixer of the Harvard Medical School. The sections were cut with a plane iron mounted between two thin pieces of wood and held in the hand. This portion of the work was done as rapidly as possible, and even then it was sometimes necessary to refreeze to keep it sufficiently hard.

The sections varied considerably in thickness, but averaged

<sup>1</sup> Manufactured by The Bausch & Lomb Optical Co., Rochester, N.Y.

about  $50\mu$ . They were removed from the plane iron with a small camel's-hair brush and transferred to dilute glycerine, to which sufficient picrate of ammonia solution had been added to give it a yellow color.

The sections were easily straightened out by means of a brush or needles with the aid of a Leitz dissecting microscope, and the best ones removed to slides and mounted in dilute glycerine with a trace of picrate of ammonia. The specimens kept very fairly and bore transportation well. If desired, the cover glass can be cemented on with zinc white or with a mixture of equal parts of hard paraffin and, turpentine free, Canada balsam applied warm with a brush. My best preparations kept for several months without marked deterioration when no cement was used. The cell outlines are much sharper when the preparation is first mounted. The cells seem to swell in the glycerine and become more transparent, which often causes the nerve fibers to appear more distinctly. The preparations vary greatly under what appear to be exactly the same conditions. This may be due to different physiological conditions of the tissues, which it is as yet impossible to determine. As with the Golgi method, there are great variations in the good preparations, some showing one detail much better than others. It is not difficult, however, to make out all of the main points of peripheral nerve distribution in nearly every well-stained preparation.

It was found that if the tissues were nearly dead before being placed in the stain, or were allowed to remain too long in the stain, or the temperature was too high during the operation, isolated epithelial cells were deeply stained, or numerous deeply stained granules appeared about the base of the hair cells or over their surface. The nerves in this case often appeared as rows of disconnected deep blue beads. If too strong a solution of the methylen blue was used, varicosities were numerous along the course of the nerve fibers, and cells were also stained deeply. A dilute solution of the stain seems to act physiologically, and the tissue is killed and fixed by the picrate of ammonia solution. When both cells and nerve fibers are deeply stained it is impossible to satisfactorily determine their relation, just as

in the case with similar Golgi preparations. Geberg found that in his best preparations the hair cells were not stained. As was independently found by both Feist and Niemack, the blood corpuscles in the capillaries may stain in a way which is quite confusing, as they contain intensely colored granules which look like the larger varicosities of the nerves.

If the fixing fluid is allowed to act too long, the epithelium is either macerated off or is so loosened that it is removed in the subsequent treatment. The osmic acid tends to prevent this maceration, but if too much is added it blackens the epithelial cells sufficiently to impair the clearness of the pictures shown. The sugar solution tends to prevent the disintegration of the epithelium or the rupture of the cells during freezing by preventing the formation of ice crystals in the cells. If the sugar is not removed from the surface of the ampullae, they are not held as firmly by the frozen gum arabic.

Bethe's method gave very good results, but for this particular tissue was not as satisfactory either in the time required or in the results obtained as the method outlined above.

The papers of Dogiel ('90) and Apáthy ('92) were freely used in working out the method which was adopted.

### III. *Innervation of Auditory Epithelium in Mustelus.*

This investigation was confined to the study of the ampullae. Large numbers of medullated nerves blackened by osmic acid were seen ascending to the cristae, but just before reaching its proximal surface the medulla disappeared. The nerve fibers could in some cases be seen through the lightly stained medullary sheath, and could be traced through the closely crowded capillaries found just inside the auditory epithelium. It was very difficult at first to do this, as the blood corpuscles were stained (as already observed by Feist, '90, and Niemack, '92) in such a way that there appeared to be an irregular mass of varicose nerve fibers crowded together at this point. The nerve fibers could be seen on both sides of it, proximally and distally. At last, as has been already stated, with more satisfactory preparations there was no difficulty in tracing the deep blue

nerve fibers through the network of capillaries, particularly at the edges of the crista, where the capillaries were less numerous and the outlines of the corpuscles could be clearly distinguished. It was apparent that deeply stained granules (Figs. 1-3, 5) in the corpuscles resembled the varicosities so closely as to be easily mistaken for them when they were closely crowded together.

The nerve fibers generally divided dichotomously, rarely into three branches. At the point of division or separation of the fibers, triangular enlargements were sometimes observed (Figs. 2, 8, 22), as has been so frequently noted by Retzius and many others. These enlargements were found to be due to the nerve sheath, which appeared to be stretched at this point (Figs. 2, 22). In many cases the nerve fibers could be distinctly seen through the walls of the sheath, and were easily followed.

The nerves branched at different levels, as observed by Retzius, but were much more numerous at the base of the hair cells, as stated by Lenhossék, than at other points. Horizontal branches were very common at the base of the hair cells, and from them branches arose, which either ended in the characteristic enlargements in contact with the basal portions of the cells, or passed between them to end free near the surface in similar but smaller enlargements. In some cases the branching fibers were so numerous as to appear to form anastomoses, but closer examination failed to demonstrate them (Figs. 1-4). A few nerve fibers were noticed which extended backward from the surface (Figs. 4, 6, 8). In other cases the fibers extended horizontally for a long distance in the middle portion of the epithelium (Figs. 2, 6).

Varicosities were very rarely seen in well stained preparations and when seen, as observed by Ayers ('93), there was no increase in the size of the nerve fiber. They appeared to be due to a semitransparent, faintly stained sheath (Fig. 22).

The lower or supporting cells, of which there were several layers, were not figured, the whole epithelium being represented in the drawings by a wash of yellow corresponding to the color produced by the picrate of ammonia. Only a few of the hair cells were drawn to show their relation to the nerve fibers. The



closely crowded hair cells were found at two slightly different levels, as shown by the position of their nuclei. The hairs of the hair cells were often lacking, but in many cases were so perfectly preserved that the individual hairs could be distinctly seen with the oil immersion lens. It was noticed that the nerve fibers often took the stain more perfectly at the edges of the crista than elsewhere, no stain being seen at any other part in some cases.

The terminal enlargement of the nerve fiber, deeply stained, was almost invariably surrounded by a clear, lightly stained vesicle, and was always present, whether the fiber showed varicosities or not. No indication of the clover-leaf (*dreilappig*) nerve termination observed by Bethe ('94) in contact with the cells was seen. The nerve endings in contact with the hair cells showed essentially the same structure as the free endings. Occasionally free endings were found in the central parts of the epithelium (Fig. 22), where the relation of the parts had not been disturbed. In some cases the nerve fibers branched at the base of the hair cell, the two parts closely adhering to the cell, and ending in enlargements at nearly the same level on opposite sides of the cell (Figs. 14, 16-18). In others the branches were not associated with the same cell (Fig. 12). Nerves ending at the very base of the cell (Figs. 15, 19, 20) were also observed. The hair cells were very rarely stained, while the nucleus was often faintly outlined, but never deeply stained.

A considerable number of cells were observed in which, if the cell had been deeply stained, it would have been impossible to prove that the nerve fiber did not enter the hair cell (Figs. 5, 9, 11, 13, 16, 17, 19, 20, 23). In cases such as those shown in Figs. 9 and 13, where the nerve ends at the very base of the cell, no continuation of the fiber into the cell could be seen. In many specimens where the nerve fiber in contact with the cell was deeply stained, an effort was made to trace a connection between them by following the nerve fiber into the cell. It was always unsuccessful, although the outer parts of the cell were semitransparent. In a single case, where the cells had become separated from their neighbors, it looked as if the fibres could

be traced into the cells (Figs. 23-25). A careful comparison of the size and relative position of these cells with others in the same section and in other preparations led to the conclusion that this was no exception to the arrangement found in other cases, and that there was no evidence of the nerve fiber entering the cell. The section cut the hair cells obliquely, the proximal end, or base, being higher than the distal end. It is almost certain that parts of two or three cells are shown, and that the nerve termination is of the same kind as that shown in Figs. 14 and 17.

It is interesting to note that the nerve endings so intimately associated with the hair cells are not in any case observed placed at a higher level than the nucleus.

In some poorly stained preparations a granular mass was observed at the base of the hair cells, but it was found that the granules were observed at different levels in other specimens, and were almost entirely lacking in sections that showed the nerve fibers most distinctly.

#### IV. *Conclusions.*

(1) In good preparations there is no trace in *Mustelus* of Kaiser's cup-like nervous mass at the base of the hair cells.

(2) The varicosities on the nerve fibers are very rare, and when present are caused by the separation of the sheath from the nerve fiber.

(3) The terminal enlargement of the nerve fibre is not like the varicosities, as it is always present.

(4) The triangular enlargements observed at the points where the nerves branch are also due to the nerve sheath, through which the nerve fibers can be seen.

(5) When the physiological effect of the methylen blue is obtained, the hair cells are not stained.

(6) The staining of a cell does not necessarily indicate that it is a nerve cell.

(7) When dying tissue is used, cells are frequently deeply stained, making it impossible to determine their relation to the nerve fibers.

(8) No satisfactory evidence of anastomosis of nerve fibers was obtained.

(9) Ehrlich's method, under suitable conditions, may give as well defined pictures as Golgi's, and leaves the cell in a better condition for studying the relation of the cell and nerve fiber, as the former is generally more transparent.

(10) No unquestionable evidence of continuity of the nerve fiber and hair cell was found.

(11) There are two kinds of nerve endings in the auditory epithelium of *Mustelus*, the greater number being free near the surface and the others in contact with the base of the hair cells.

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## DESCRIPTION OF PLATES.

The auditory epithelium is colored yellow. The nerve fibers are violet, and the medullary sheath neutral tint. The capillaries are violet of different tints, due to the variously stained blood corpuscles which fill them.

*Lettering used uniformly throughout the figures.*

*m.* = medullary sheath.

*c.* = capillaries.

*n.f.* = nerve fibers.

*n.* = nuclei.

*t.e.* = terminal enlargement.

*h.* = terminal hairs.

*h.c.* = hair cells.

All drawings were made with Abbé camera lucida on Bernhard's drawing table, to avoid distortion.



## EXPLANATION OF PLATE VII.

FIG. 1. Transverse section of crista acustica, showing medullated nerves, nerve fibers branching in the epithelium and the capillaries at the base of the epithelium. Zeiss, oc. 4, obj. A.

FIG. 2. The auditory epithelium, showing arborization of nerve fibers and relation of nerve terminations to auditory or hair cells. It also shows the granular effect produced on blood corpuscles in the capillaries. A single medullary sheath is shown in cross section. Zeiss, oc. 4, obj. D.

FIG. 3. This section of the auditory epithelium shows arborization of nerve fibers, many nerve terminations, and a single hair cell. Medullated nerves and capillaries are also shown. Zeiss, oc. 4, obj. D.

FIG. 4. An unusual branching of nerve fibers in the auditory epithelium. Two nerve terminations are shown near the outer surface. Zeiss, oc. 4, obj. D.

FIG. 5. A portion of the crista acustica, showing near its edge nerve fibers and a single hair cell; also capillaries and a single medullated nerve. The nerve fiber is visible through the sheath. Zeiss, oc. 4, obj. D.

FIG. 6. A single nerve fiber and its branches. Zeiss, oc. 4, obj. D.

FIG. 7. This section shows a bundle of nerve fibers as it penetrates the basement membrane and the fibers separate in the auditory epithelium. A single hair cell is shown. Zeiss, oc. 4, obj. D.

FIG. 8. Method of branching of nerve fibers and relation to hair cells. Triangular enlargements where nerves branch. Zeiss, oc. 4, obj. D.

FIG. 9. Two nerves and the relation of their branches to hair-cells. Zeiss, oc. 4, obj. D.

FIG. 10. A single hair cell and its relation to a nerve fiber. Zeiss, oc. 4, Leitz oil im.  $\frac{1}{12}$ .

FIG. 11. Two deep-lying cells containing numerous granules, deeply stained, and their relation to nerve fibers. Zeiss, oc. 4, Leitz oil im.  $\frac{1}{12}$ .













## EXPLANATION OF PLATE VIII.

FIG. 12. A single nerve fiber and the relation of its terminations to the hair cells. Zeiss, oc. 4, Leitz oil im.  $\frac{1}{2}$ .

FIG. 13. A single hair cell and its relation to a nerve fiber. Zeiss, oc. 4, Leitz oil im.  $\frac{1}{2}$ .

FIG. 14. Two terminations of a single nerve fiber at the base of a hair cell. Zeiss, oc. 4, Leitz oil im.  $\frac{1}{2}$ .

FIG. 15. Shows the relation of a nerve fiber to a hair cell. Zeiss, oc. 4, Leitz oil im.  $\frac{1}{2}$ .

FIGS. 16, 17, 18, 19, 20, and 21 all show the relation of nerve fibers to hair cells. Zeiss, oc. 4, Leitz oil im.  $\frac{1}{2}$ .

FIG. 22. A nerve fiber with its branches. Also two transparent vesicles (varicosities) surrounding one of the fibers and triangular enlargement where the nerves branch. Zeiss, oc. 4, Leitz oil im.  $\frac{1}{2}$ .

FIG. 23. A surface view of nerve fiber and its relation to hair cells. Zeiss, oc. 4, Leitz oil im.  $\frac{1}{2}$ .

FIG. 24. The same cells focussed to show deeper portions of the cells. Optical section.

FIG. 25. Still deeper in the same cells. The nerve fiber soon passes out of the field.







## THE EPITHELIUM OF THE SO-CALLED MIDGUT OF THE TERRESTRIAL ISOPODS.

J. PLAYFAIR MCMURRICH.

THE ease with which preparations may be obtained, and the large size of the constituent cells have brought it about that the epithelium of the so-called midgut of the terrestrial Isopods has not unfrequently been the subject of observation. From its cells, within recent years, the Belgian school of cytologists have endeavored to deduce the structure of the nucleus and the cytoplasm, and it has also figured largely in the discussions on the occurrence and significance of amitosis. Still more recently Ryder and Miss Pennington have described the occurrence of nuclear conjugation in the intestinal epithelium of *Porcellio*, and it was the appearance of this paper that especially aroused my interest in the tissue, although my attention had already been directed to it by what I had seen of it during the progress of my studies on the embryology of the Isopods.

### I.

My observations have been confined mainly to terrestrial Isopods, one species of each of the genera *Armadillidium*, *Porcellio*, and *Oniscus* forming the principal sources from which my material was obtained. Preparations of the intestine of *Idotea robusta* were also made, but beyond this I have made use, so far as the present paper is concerned, of no other marine or aquatic forms.

The simplest method of obtaining the material is to immerse an animal in fluid and, inserting a needle into either extremity of the body, pull it to pieces. The "midgut" usually breaks just behind the region where the liver cæca communicate with the digestive tract, and with very little further trouble it may be isolated almost in its entirety. If a surface preparation were required, the intestinal contents, usually present in animals

which have not been compelled to fast for some time, were washed away, the gut was then opened lengthwise with fine scissors and spread out upon a glass slide, where it was subjected to the action of the fixing reagent. Ide's method of fixing the animal on a block of paraffin and carefully dissecting out the entire digestive tract was also used, but the more rapid method was found to be quite as satisfactory for the purposes I had in view.

The fluid in which the animal was immersed was either water, normal salt solution, or corrosive sublimate; the last being used only for the purpose of control. For fixing I employed corrosive sublimate, Hermann's and Flemming's fluids, and found all three equally good, though I made use mainly of corrosive sublimate. In staining, various reagents were employed; for flat preparations alum cochineal and alum carmine were very satisfactory, but Delafield's hæmatoxylin, well washed out with acid alcohol, proved most so. Sections were also stained with these reagents and with Haidenhein's iron-lack hæmatoxylin, which gave excellent and most instructive preparations. The Biondi-Ehrlich stain was also used, and gave valuable information as to the nuclear constituents, and Korschelt's combination of borax carmine and bleu de Lyon also proved useful.

## II.

Before entering upon the description of the epithelium it will be necessary to explain two terms that will be used. What will be meant by the *midgut* is that portion of the digestive tract which extends from the point where the liver cæca open to the beginning of the rectum. The term *midgut* is usually applied to a portion of a digestive tract which has an endodermal origin; the Isopod "*midgut*," however, is the anterior part of the procotodæal invagination, and is not, therefore, entitled to be termed a *midgut*. It seems, however, more convenient to use the term *midgut* in the following pages than to invent a new term or employ a cumbersome periphrasis, but to avoid possible misunderstanding I shall use the term only within quotation marks.

The other term whose use I wish to explain is the word *cell*.

It will be seen later that no true cell boundaries exist in the "midgut" epithelium of adult forms; indications, however, of the extent of cytoplasm which comes under the influence of each nucleus, if I may use such an expression, are to be found, and to each of these "spheres of influence" I shall apply the term cell.

The "midgut" in the terrestrial Isopods pursues a practically straight course from the stomach to the rectum, and may, for convenience, be divided into the four regions which are recognized by Ide ('92). The most anterior of these regions is characterized by possessing along the dorsal mid-line a  $\perp$ -shaped ridge which projects into the lumen of the intestine, and may be termed the dorsal ridge. The second portion differs from the first only in lacking this ridge, while the third portion is characterized by being provided with strong circular muscles, which act as a sphincter. The fourth portion, which may be termed the transitional region, is short, and communicates posteriorly with the rectum.

Throughout the entire length of the gut the general structure is the same. Its interior is lined throughout by a moderately thick layer of chitin (Pl. IV, Fig. 5, *ch*), below which is a single layer of large cells which produce the chitin and have been spoken of as the hypodermis. Beneath this layer is a basement membrane (Fig. 5, *bm*) which separates the hypodermis from the mesodermal tissues, muscles (*m*), and coelomic cells, which form the external layer of the gut.

In examining a surface preparation of the second portion of the gut (Figs. 1, 3), one sees the large nuclei of its epithelium arranged in very definite longitudinal and transverse rows, the regularity of their arrangement in the longitudinal direction being especially striking. The longitudinal rows are continued without interruption into the ventral surface and sides of the first portion of the gut, but upon the dorsal surface a number of the rows on each side of the mid-line converge towards the posterior extremity of the dorsal ridge, as is well shown in the figure given by Ide ('92, Pl. II, Fig. 19) of a portion of the "midgut" of *Oniscus*. This figure and the sections which the same author represents in Pl. III of his paper show with



sufficient accuracy the structure of the dorsal ridge. In the sphincter region the epithelial cells seem at first sight to differ considerably from those of the more anterior regions; they are somewhat more columnar, and the entire epithelium is thrown into numerous folds. These peculiarities, however, are probably to be explained by the presence of the strong sphincter muscle, which diminishes materially the lumen of the gut, pressing the epithelial cells together and throwing the layer into folds. In fact, it seems probable that the actual surface of the epithelial layer of a given length of this portion of the gut is equal to that of a similar length of the second portion, an idea which is borne out by the fact that in very young specimens, such as have been but recently set free from the brood pouch, no difference can be detected between the posterior and anterior portions of the gut, the cell rows being continuous from the rectum forward.

I have not paid much attention to the transitional region, and can only say that its cells seem to resemble closely those of the second portion of the gut, and diminish in size posteriorly rather abruptly to become continuous with the small-celled epithelium of the rectum.

From this brief sketch of the general structure of the "mid-gut," we may now pass to a detailed consideration of the structure of its epithelial cells.

The layer of chitin, which covers the inner surface of the epithelium, consists of two layers (Fig. 5), an external denser and more refractive layer (*ch'*), which stains deeply with Haidenhain's hæmatoxylin, and a less dense inner layer (*ch*), which rests on the surface of the epithelial cells and remains unstained in preparations treated with the iron-lack hæmatoxylin. *Both these layers are perfectly homogeneous, showing no trace of pores, or even of a radial structure.*

The basement membrane (*bm*), on which the epithelium rests, is very thin, and stains with the iron-lack hæmatoxylin. For the most part it is of very even thickness, but opposite the lines of junction of adjoining cells it is very frequently thickened somewhat, and below the elongated cells which are found on either side of the dorsal ridge, small thickenings are to be

seen at intervals along it. Occasionally nucleus-like structures may be seen in the thickenings in carmine preparations, and it seems probable that these are coelomic cells which have wandered into the thickening from the exterior.

The chief interest in the thickenings, however, lies in the fact that they form the bases of support of a number of peculiar fibres, or columns, which extend inwards from them through the substance of the epithelial cells (Fig. 5, *sf*). Huet ('83) has described these fibres in the following words: "Cependant on voit alors qu'elle (la membrane chitineuse) envoie au travers de celle-ci (la couche épithéliale) dans les intervalles même qui séparent les éléments épithéliaux de petits tractus, de petites colonnes qui l'unissent à la tunique conjonctive propre." He apparently considers the columns to be chitinous in structure, describing them as appertaining more especially to the chitinous membrane. Ide ('92), however, takes strong exception to this view, maintaining that they are protoplasmic and are merely "portions fortifiées du réticulum général, des séries de trabécules placées bout à bout et fortement épaissies."<sup>1</sup>

With such widely divergent opinions before us it becomes of interest to have some further light upon the character of these fibres, and this seems to be furnished by Haidenhain's iron-lack hæmatoxylin. In preparations treated with this stain the fibres stand out very clearly indeed, as they resist the decolorizing action of the iron ammonium sulphate much more perfectly than does the cytoplasm. When the decolorizing is carried to such an extent as almost to deprive the cytoplasm of the stain, the fibres appear as deeply blue-black strands extending from the basement membrane to the chitinous cuticle. They occur especially abundantly towards the periphery of the cells, though they are by no means confined to that region, but very frequently may be seen passing through the cytoplasm in close proximity to the nucleus. Huet ('83), as has been stated, considered them to arise from the chitinous cuticle, but it seems more correct to describe them as arising from the basement membrane, from which they spring either singly or, more frequently, in groups,

<sup>1</sup> I have not had access to the work in which Leydig mentions these fibres. I know of it only through a remark contained in Ide's paper.

in which case there is generally a thickening of the basement membrane at their point of origin. From the thickenings of the basement membrane, which occur opposite the lines of junction of adjacent cells, numerous fibres always arise. At their origin the fibres are of considerable strength, but as they pass towards the cuticle they split lengthwise and finally fray out into fine fibrils which are attached to the under surface of the cuticle. These final portions of the fibrils do not hold the stain as do the fibres proper and their larger branches, but resemble in this respect the lower layer of the chitinous membrane with which they are connected.

In very young specimens of *Porcellio* and *Armadillidium*, no trace of the supporting fibres could be discovered, and in a specimen of *Oniscus* measuring 4 mm. in length they were but slightly developed, projecting into the cytoplasm from the basement membrane but a short distance (Fig. 11, *sf*). Preparations from the "midgut" of adult specimens of *Idotea robusta* showed practically the same conditions as the 4 mm. specimens of *Oniscus*.

As I have pointed out, the behavior of the fibres to iron-lack hæmatoxylin indicates for them a different chemical composition from the cytoplasm, and it is to be noted that it also indicates for them a similarity to the basement membrane from which they arise. Their terminal fibrils, however, appear to partake rather of the character of the chitinous membrane, but it is presumable that they are altogether products of the cytoplasm, and not, as Ide supposes, simply thickened portions of the cytoplasmic reticulum. A confirmation of this idea, derived from their chemical behavior, is furnished by a physical character which they possess, namely, *brittleness*. Sections frequently cut the fibres at an angle, and in such cases they are often broken across, the lines of fracture being clean and distinct, as is shown in Fig. 5, *sf'*. This brittleness is probably the result in part of the reagents used in imbedding, but it is a character quite foreign to protoplasm when similarly treated, and one frequently observable in chitinous and certain supportive substances, such as, for instance, the mesogloea of some Cœlentera.



It would seem, then, that the fibres were partly chitinous and partly of a substance similar to that which forms the basement membrane. This double structure will not seem anomalous when we consider that the epithelial cells manufacture on their two surfaces substances which are chemically distinct; on their outer surface the basement membrane, and on their inner surface the layer of chitin. That the basement membrane is formed by the epithelial cells and not by the mesoderm is indicated, it seems to me, on the one hand by the perfect continuity of the basement membrane and by the manner in which it moulds itself over the bases of the cells and frequently is thickened at the point of contact of two or more cells, and on the other hand by the absence of a definite layer of mesodermal cells on the outer surface of the gut.<sup>1</sup> We need not be surprised, then, to find that the fibres, in those portions of their extent which traverse the regions of the cytoplasm which have manufactured the basement membrane, are of the same material as that structure, while those portions of them which traverse the chitinous regions of the cytoplasm are chitinous in character.

The occurrence, however, of what, for convenience, may be termed supporting fibres traversing the cytoplasm is somewhat unusual, chitin and the basement membrane substance being usually found in layers resting upon or underlying epithelia. A certain peculiarity of structure found in the Crustacea is, however, of some interest in this connection, namely, the insertion of the muscle tendons into the exoskeleton. This is accomplished by means of tendons which, according to the observations of Reichenbach ('86), have an ectodermal origin in *Astacus*. It will be conceded that the peculiar mode of insertion of the extrinsic muscles of the œsophagus and rectum of the Isopods, described by Ide ('93), in all probability has a similar origin.

<sup>1</sup> I have in another place (*Text-book of Invertebrate Morphology*, New York, 1894) insisted upon the homology of the basement membrane, which exists between the ectoderm and mesoderm of the Turbellaria, and the mesoglœa of the Cœlentæra, which, as is well known, is primarily a formation of the epithelial cells, — the mesoglœal cells, when they occur, only secondarily migrating into it. In the Isopod "midgut" we have to deal with an arrangement similar to that found in the Turbellaria, since the gut is of ectodermal origin. The occurrence of cœlomic cells in the thickenings of the basement membrane of the Isopod "midgut" has additional interest in this connection.

The tendons pass between, and apparently in some cases (see Ide's Fig. 64) through, the epithelial cells of the intestine, to be inserted into the chitin lining the interior of the gut; and it may reasonably be assumed that they are produced by cells which were at one time, at least, a portion of the epithelium of the œsophagus or rectum. If the ectodermal cells of the stomodæum and of the terminal portion of the proctodæum can produce these tendinous structures, we can readily understand how the cells of the anterior portion of the proctodæum, since this is in reality the true significance of the "midgut," may produce the fibres.

Since the above lines were first written I have received, through the courtesy of the author, a paper by Bergh ('96), in which he describes the occurrence of supportive fibres in certain Infusoria. How far they agree with the fibres I have just discussed remains to be seen, but it is nevertheless interesting to find intracellular supporting fibres in other forms than the Isopods.

*The Cytoplasm.*—In examining a surface view of the "midgut" (Fig. 1), each of its large nuclei seems at first sight to be approximately in the centre of a more or less quadrangular area of cytoplasm, each area being separated from its neighbors by well-defined boundaries. On further examination, and on altering the focus to a deeper level so that the bases of the areas are brought clearly into view, the boundaries almost disappear,

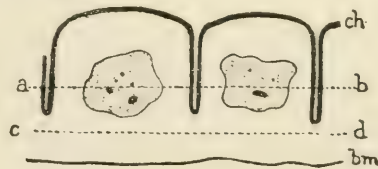


FIG. 1.

and the impression that one has to do with rows of distinct cells becomes more feeble. This results from the fact that the areas, or cells, are somewhat dome-shaped, and the chitinous cuticle is moulded over the surfaces of the domes, as is represented in the annexed diagram. Consequently, when the middle of the cell and its nucleus are in focus, as when the optical sec-



tion is in the plane *a b* of the diagram, one looks down on the edges of the depressions of the cuticle between the adjacent domes, and so obtains an optical transverse section of the cuticle, while by focussing lower, as in the plane *c d* of Fig. 1, the lower edges of the depressions of the cuticle are passed and the cells appear less distinctly separated.

I wish to insist upon this difference in the appearance of the optical section according as the focus is high or low, since I believe that a failure to note the true significance of the apparent boundaries has led certain authors to an erroneous interpretation of one of the peculiarities of the epithelial nuclei. To this I shall return later on, but here I may point out that Carnoy ('84) has evidently fallen into the error. Thus he says, "En dehors du protoplasme à la limite des cellules, se dessine une zone brillante, divisée en son milieu par une ligne plus sombre. Celle-ci n'est autre chose que la *membrane primaire*, commune aux cellules juxtaposées; tandis que les lamelles blanches qui la bordent représentent la *membrane secondaire*, qui est propre à chaque cellule et s'est formée plus tardivement." As a matter of fact, Carnoy's "membranes secondaires" (see Fig. 2, *ch*) are simply the layers of chitin as they dip down between the domes of adjacent cells, while his "membrane primaire" is probably merely the interval between the two folds of chitin!

The distinctness of the epithelial cells being then only a superficial appearance, is it to be believed that the lack of distinctness towards the bases of the cells is due to the actual continuity of the protoplasm throughout the gut? Huet ('83) supposes that such a continuity exists, and I believe that the evidence presented by sections bears out his supposition. Never have I seen in preparations from adult individuals any such distinct and regular cell boundaries as are figured by Ide ('92), but in those situations where, in sections, one would expect to find the cell walls, one sees only the supportive fibres, and these, not being lamellæ, allow the protoplasm of adjoining cells to pass between them and become continuous.

In preparations from young specimens, however, a distinct separation between adjoining cells seems to be present, and it is probable that the individuality of the cells is present in young

specimens, but is lost in the adults; a change which is probably correlative with decided alterations in the structure of the cytoplasm.

The cytoplasm in young specimens has a uniform, finely reticulated structure (Fig. 11), but this is replaced in the adults by a much less uniform arrangement. In addition to the development of the supportive fibres which traverse it, the protoplasm has lost to a certain extent its reticular structure, becoming more granular; and, at the same time, vacuoles (Figs. 4, 5, *v*) have developed in it in considerable numbers. Towards the outer and inner surfaces of the cells the reticulum is more apparent than elsewhere, but I have seen cells whose central parts were practically a single large vacuole, only the traversing fibres being surrounded by protoplasm.

In all the land forms I have examined, the cytoplasm is for the most part in close contact with the under surface of the chitinous cuticle, but in nearly every surface preparation places can be found where there is a distinct space between the two, this space being, however, traversed by the supporting fibres and, apparently, also by strands of protoplasm. In surface views, with a high focus, these strands are seen passing on all sides to the folds of chitin between the domes of adjacent cells, and one gets an appearance as if there were intercellular bridges of protoplasm connecting the cells. This appearance vanishes, however, when the focus is set at a lower level, and sections (Fig. 5) demonstrate its true significance. In *Idotea robusta* this structure was particularly evident (Fig. 6), being present in every cell, and here the strands traversing the space are probably protoplasmic alone, since the supporting fibres project up into the cytoplasm only a short distance from the basement membrane.

In addition to the vacuoles which have already been mentioned and which possess fluid contents which do not stain with the reagents employed, others of a different character occur in adult animals, though I have not found them as extensively developed in either *Porcellio* or *Oniscus* as in *Armadillidium*. In the last-named genus the vacuoles, which are characterized by containing a floccular stainable material, extend frequently

through many cells (Fig. 12, *v*), appearing in surface preparations like more or less extensive blisters of the epithelium. I at first thought they were artifacts produced by osmosis during the preparation of the gut for fixation, but preparations which were made from animals opened under corrosive sublimate, so that fixation of the tissue took place before any osmosis capable of producing such changes could occur, showed them quite as well as preparations from animals which were opened under normal salt solution. They undoubtedly exist in the living epithelium. Sections (Fig. 12) through a large vacuole of this type show that it is contained within the cytoplasm, the outer and inner layers of which are forced apart, the nuclei of the cells lying sometimes in the outer and sometimes in the inner layer, and usually showing greater or less signs of compression. The supporting fibres in the area covered by the vacuole are broken across.

In none of the literature to which I have access have I found the slightest reference to these blister-like vacuoles. The shaded area shown on the left side of Ide's Fig. 19 ('92) looks as if it might be one of them, but no reference is made to it in the text, and in the description of the figure it is said to represent an area occupied by cells of a more columnar form than are found elsewhere. As has been noted, however, these vacuoles are of much less frequent occurrence in *Oniscus* and *Porcellio*, the genera most abundantly studied, than in *Armadillidium*. In *Idotea* vacuoles extending through two cells were very abundant, but I found none at all comparable in extent to those of the land genera.

Other products of metabolism are not abundant in *Oniscus* and *Porcellio*; occasionally one or two large spherical granules which stain a deep-red color with the Biondi-Ehrlich stain are to be found in some of the cells, and are probably metabolic products. In *Armadillidium*, however, the case is very different. In fully grown specimens of this genus very numerous granules of a greenish-yellow color are frequently found in every cell (Figs. 3, 5). They are scattered to a certain extent through the cytoplasm, but are always much more densely aggregated immediately below, *i.e.*, external to the nucleus, a



fact which recalls the ideas expressed by Korschelt ('89), as to the influence of the nucleus in cell metabolism. These granules are not, however, always present even in specimens which seem to be fully grown, but when they are present at all they occur throughout the entire extent of the first and second portions of the "midgut." It seems probable that the specimens in which they occur are older than those which, though approximately of the same size, lack them.

*The nucleus.* — The nuclei of the "midgut" cells of the terrestrial Isopods have attracted considerable attention on account both of their large size and of the peculiar forms which they assume. Their most usual form is that of an oval, but this is rarely symmetrical (Fig. 1); very frequently the nuclei are lobate or provided with numerous blunt processes giving them the appearance of having been amoeboid during life; and in some cases branched nuclei, resembling in shape those of the silk glands of the Lepidoptera, may be found, and others of most bizarre shapes are not uncommon.

It will be unnecessary to figure the various forms which occur, since van Bambeke ('87) has shown many of them in his article on the artificial deformation of the nucleus. For these observations the intestine, taken from the living animal, was rapidly torn to pieces, and thereafter stained with an acid solution of methyl green. After this treatment nuclei of remarkable shapes were frequently seen, and the conclusion was drawn that the irregularities were due to the mechanical injuries the nuclei had sustained. At the same time the author points out that sometimes, even when the treatment of the intestine has been most severe, no deformed nuclei can be observed; and *vice versa*, when care was taken to avoid injury of the tissue the deformed nuclei were sometimes abundant. I have not attempted to distort the nuclei in any of my preparations, but on the other hand have been as careful as possible to avoid injuring the tissue, and I find that in different specimens, treated as nearly as possible with the same care, sometimes nearly all the nuclei are extensively deformed, and at other times none of the more pronounced deformities can be observed (*cf.* Figs. 1, 3). I mention this fact in evidence of the supposition that many of

van Bамbeke's deformed nuclei were not produced by the mechanical insults to which he subjected the tissue, but were already present in the living intestine. It is true that highly deformed nuclei are frequently found where in a preparation there has been a slight laceration of the walls of the intestine, —indeed, I have found the most extensive deformations only in such situations; and granting, as there is reason to do, the viscous character which van Bамbeke assigns to the nuclear contents, there is no reason for doubting that deformations may be caused by rough treatment. But on the other hand the facts which I have stated above seem to me to show that in the Isopod "midgut" we have to do with nuclei capable of extensive alterations of shape.

These alterations in shape naturally produce in many cases constriction of the nucleus, and with the proof of the occurrence of amitotic division the supposition became admissible that constricted nuclei were nuclei in process of amitosis. Carnoy ('85) devotes a brief section to the amitosis of the intestinal cells of the Isopods, but unfortunately he gives no detailed account of the nuclei on which he bases his conclusions as to the occurrence of the phenomenon, but simply refers the reader to figures of cells from the testis which "*représentent exactement ce qui se voit dans le noyau des cellules intestinales quant au phénomènes de la division.*" Though I have examined many preparations of the intestine of the land Isopods, I have never seen any nuclei which I could say were *exactly* similar to Carnoy's figures; and though I have found many constricted nuclei, I have never been able to satisfy myself that normal amitosis actually occurred. Ziegler and vom Rath ('91) also maintain the occurrence of amitosis in the Isopod "midgut," but do not give any detailed evidence in support of their contention.

When I first began the study of the Isopod intestine I interpreted the lobed and constricted nuclei which I saw as stages of amitotic division, but further observation brought doubts as to the correctness of such an interpretation. The extreme variety of form which occurred seemed to point rather to amoeboid alterations of shape than to division; and the fact that, notwithstanding the enormous number of constricted nuclei



that I saw, I could never find cases in which the daughter nuclei were completely formed, but still in contact with one another, awakened suspicion. The arrangement, too, of the nuclei in such definite, longitudinal, and transverse rows seemed hardly in harmony with the occurrence of such frequent amitoses as the irregularities of the nuclei suggested; but, on the other hand, the occurrence of multinuclear cells seemed to be a point in confirmation of the occurrence of amitosis. In the land Isopods this condition is found rather infrequently, but it seems more common in marine forms. Thus cells with two nuclei are of rather frequent occurrence in *Idotea robusta* (Fig. 6), and Carnoy states that in *Cirolana* nearly every cell contains from ten to thirty perfectly formed nuclei. Further consideration of this point led me again, however, to doubt the advisability of attaching much importance to it. In the land Isopods the cytoplasm over each nucleus is, as a rule, elevated more or less into a dome over which the chitinous membrane is folded; if, now, occasionally two cells unite to form a single dome, we would have apparently a cell with two nuclei, and it is possible that this may have been of frequent occurrence in *Idotea*, and that groups of cells have united together to form the supposed multinucleated cells of *Cirolana*.

This explanation of the occurrence of the multinucleated cells is, I think, a possible and a plausible one. But let us consider on general grounds the probability of the occurrence of such extensive amitoses as the irregular form of the nuclei would lead us to expect, assuming that the irregularities indicate this phenomenon. We can imagine cell-division, amitotic or mitotic, taking place in the "midgut," either for growth or for regeneration. To determine whether the growth of the "midgut" depended on cell-division or on the increase of size of the various cells, I made preparations of the gut of a series of specimens of *Oniscus* of different sizes, the largest specimen measuring 12.5 mm. in length, and the smallest 4 mm. In each preparation I measured the length and breadth of a number of cells and took the average of the measurements; the results are represented in the following table:

LENGTH OF SPECIMEN.	AVERAGE LENGTH OF CELLS.	AVERAGE BREADTH OF CELLS.
4 mm.	26.6 $\mu$	27.7 $\mu$
6 mm.	33.4 $\mu$	39.5 $\mu$
9 mm.	47.5 $\mu$	54.7 $\mu$
12.5 mm.	69.9 $\mu$	104.9 $\mu$

We see from this that there is a gradual increase in the size of the cells in passing from the 4 mm. specimen to the 12.5 mm. one. Whether this increase in the size of the cell is sufficient in itself to account for the increased length of the "midgut," it is difficult to estimate, as I did not succeed in isolating the entire "midgut" in the smaller specimens, and therefore could not determine its length. If we take the length of the entire animal as the basis of comparison, a certain allowance for the stomodæum and the rectum ought to be made, but how much I cannot say. However, even without making any allowance, the figures obtained by such a comparison have considerable interest, and I give them for what they are worth. The length of the body of the smallest specimen is taken as the unit, and with it is compared the length of the body of each of the other specimens, and similar comparisons are made between the lengths of the cells of the various specimens and that of the cells of the smallest specimen, which are again taken as a unit.

COMPARISON MADE BETWEEN	LENGTH OF BODY.	LENGTH OF CELLS.
4 mm. and 6 mm.	1 : 1.5	1 : 1.26
4 mm. " 9 mm.	1 : 2.25	1 : 2.18
4 mm. " 12.5 mm.	1 : 3.1	1 : 2.6

We find here, even without the corrections that should be made, evidence that the increase of length of the "midgut" is produced by an increase in the length of the cells which compose it, and not by the formation of new cells.

With regard to the increase in the circumference, evidence is furnished not only by the measurements but also by simply

counting the number of cells seen in transverse sections. This I have done with specimens of three ages; the youngest had just been set free from the brood pouch, and the other two were the 4 mm. and 12.5 mm. specimens, from which the measurements were made. The number of cells found in a transverse section of each of these is as follows:

Just set free	4 mm.	12.5 mm.
33	30	38-40

The figures do not agree absolutely, but since the smallest specimen has a greater number of cells than the 4 mm. one, it is quite probable that the differences are individual differences and do not denote an increase in the number of cells in the intestine as the individuals grow older.

The evidence, then, which I have presented, though not absolutely conclusive, points very strongly, it seems to me, to the supposition that *the increase in the size of the "midgut" of the land Isopods is not due to an increase in the number of cells, i.e., to cell-division, but to an increase in the size of the cells present at the close of embryonic life.*

The last part of this statement is an anticipation, and brings us to the question of regeneration. I have nothing to add to the discussion as to whether regeneration can take place by amitosis, but may simply state that I do not believe that regeneration occurs in the forms which I have studied. In the first place, the gradual increase in the size of the "midgut" cells as the animals grow older does not favor the idea that the cells are thrown off from time to time and replaced by new ones, and in the second place regeneration is usually associated with cells which possess a glandular function, and of these there is not the slightest trace in the Isopod "midgut." Ziegler and vom Rath ('91) describe mitoses at the beginning of the "midgut" of an adult *Anilocra*, and regard them as producing regeneration; but before this idea can be accepted it must be shown more definitely that these mitoses are not associated with the regeneration of the cells of the liver cæca, and, furthermore, it must be shown that the region in which they were found was really the ectodermal "midgut" and not a

slightly developed true endodermal "midgut." At all events the cell-divisions described were mitoses and not amitoses, and no trace of regeneration by amitotic division has yet been found in the Isopod "midgut."

I do not wish it to be understood that I am denying the existence of amitosis in one form or another in the "midgut" of the land Isopods; my point is that it cannot occur by any means as frequently as has been supposed, and that the peculiarly formed nuclei are not necessarily nuclei which are undergoing amitosis. I have myself observed certain arrangements of nuclei, which I have represented in Figs. 7 and 8, and which seem to be explicable only as the result of a direct division of the nucleus; they represent, however, a *fragmentation* of the nucleus rather than what is usually denoted amitosis. In some cells one or two small portions of nuclear substance may be found lying beside a nucleus, as is shown in Fig. 7, and in one instance (Fig. 8) I observed the results of the fragmentation of a nucleus into a number of small portions. Cases such as these I have found only in fully adult specimens, and I believe them to be the results of beginning disorganization rather than a multiplication of nuclei.

It is interesting to find that the Isopod "midgut" has been held to furnish evidence not only of the occurrence of amitosis, but also of a reverse and more remarkable process, namely, a conjugation of nuclei. The late Prof. J. A. Ryder and one of his students, Miss Mary E. Pennington, have described ('94) such as occurring in the "midgut" of *Porcellio*. The figures which they give to substantiate their conclusions may readily be duplicated, and in Fig. 3 I show some instances of the same phenomenon as they describe. But the interpretation of such peculiarities as a fusion of nuclei seems to me to be entirely unwarranted. Their mistake has arisen from a misconception of the true structure of the "midgut" epithelium; they have evidently regarded, just as Carnoy has done, the appearances seen with a high focus, to which I have referred in an earlier part of this paper, as denoting a perfect separation of the various cells, and have failed to perceive that we have really to do with a syncytium.



A. B. Lee ('95) has taken exception to the interpretation given by Ryder and Miss Pennington, but has erred quite as greatly as the original authors in the explanation which he gives. He supposes the bands of nuclear substance, which they have figured as extending from one cell across the boundary into another, to be persistent nuclear spindles. The examination of a single preparation of the "midgut" of a land Isopod which showed the phenomenon, and they are not difficult to obtain, would have shown Lee at once that his conclusion was an untenable one, and he might have saved himself from a too hasty generalization. The bands are undoubtedly nuclei, and resting nuclei at that, staining intensely with the usual nuclear stains, and not requiring the use of the Kernschwarz for their unmistakable demonstration.

The true significance of this supposed nuclear conjugation, which after all is based merely on the occurrence of nuclei which pass across the apparent boundary between two cells, may be understood when we recall two peculiarities of the "midgut" epithelium, namely, that it is a syncytium, and that its nuclei are capable of extensive amœboid movement. These movements are undoubtedly at times quite vigorous, if we may judge from the remarkable shapes which the nuclei present; and there being no true cell boundaries, there is no mechanical obstacle in the way of one nucleus invading the territory of another. This, I believe, is simply what happens, and there does not seem to me to be the slightest ground for assuming that any such remarkable process as a nuclear fusion takes place in the adult and remarkably modified cells of the "midgut" of the terrestrial Isopods.

A few words yet remain to be said concerning the minute structure of the nucleus. A well-defined membrane surrounds every nucleus (Figs. 2, 10, *nm*), and the nuclear contents seem to be composed of four substances. In the first place there is a considerable amount of caryolymph, not infrequently aggregated to form intranucleolar vacuoles, a peculiarity especially noticeable in one preparation I possess from *Idotea robusta*, two cells from which are represented in Fig. 6. In nearly every nucleus the caryolymph seems more abundant towards the



periphery, a relatively clear zone with but little of the other constituents being found immediately within the nuclear membrane (see Fig. 4).

Secondly, there is the chromatin, which in the less-deformed nuclei is in the form of innumerable spherical granules (Fig. 10), more densely aggregated towards the centre of the nucleus (Fig. 2), but occurring also in less numbers in the clear zone just within the membrane. The size of the granules seems to vary somewhat in different specimens, so that the nuclei present in some cases a relatively coarsely granular appearance. Carnoy ('84) and van Bambeke ('87) maintain the opinion that the chromatin is arranged in a skein in these nuclei; I have seen nothing which would lead me to support this view, the granules being perfectly separate from one another and scattered irregularly through the nucleus. In the most highly deformed nuclei a greater or less portion of the chromatin loses its granular arrangement, the granules being apparently drawn out so as to assume the form of fibrils, and sometimes fusion of the various fibrils seems to occur, so that portions of the nucleus appear practically homogeneous. Van Bambeke ('87) has noted these peculiarities and concludes that the chromatin in the living cells has a viscous consistency, a view which there seems no reason to doubt.

Thirdly, the chromatin granules are supported upon an achromatic substance (Fig. 10), which is not easily demonstrable owing to the number and dense arrangement of the granules. It seems, however, to possess a reticular arrangement.

Finally, nucleoli (Figs. 2, 10, *nl*) are to be found in all cells, varying from a size almost equal to that of the chromatin granules to several times that size, and apparently varying also in number from two to several. In *Idotea robusta* the nucleoli seem to be of an almost constant size, and never exceed two in number.

At first sight the nuclei remind one of the figures, given by Korschelt ('96), of the branched nuclei of the silk glands of the Lepidoptera; and one might suppose that what I have termed the chromatin granules were really what Korschelt has termed microsomes, and that the nucleoli were the real chromatin

masses. The large size of the chromatin granules, as compared with the microsomes, and their relation to the achromatic substance throw doubt on this idea, however, and the doubt is confirmed by the behavior of the nuclei to the Biondi-Ehrlich stain. With this Korschelt found the microsomes to assume a red color, and the chromatin masses became green; in the Isopod nuclei the granules took the greenish hue, while the nucleoli became red. It seems that the granules of the Isopod nuclei are equivalent to the chromatin masses of the silk glands, while the nucleoli of the Isopod nuclei are not represented in the glands, and the microsomes of the latter do not occur in the Isopod nuclei.

The granular arrangement of the chromatin is perhaps the most striking feature of these nuclei, and it is one which develops early. In the embryos, up to the time when the formation of the digestive tract is completed, the nuclei possess the structure usually seen in embryonic tissues, but in individuals which have just left the brood pouch the transformation of the nuclear structure has already begun. In Fig. 9 is shown a nucleus from such a young individual; one sees the chromatin in the form of granules, scattered on the achromatic reticulum, and it is noticeable that in addition to several large granules numerous small ones occur. In other nuclei the number of small granules was much larger, and in specimens 4 mm. in length (Fig. 11) the granular arrangement was almost as fully developed as in the adults. I may state that I have not, in adult cells, been able to distinguish centrosomes or archoplasm spheres.

### III.

In the epithelium of the "midgut" of the terrestrial Isopods we have a tissue which possesses many peculiarities, the most striking of which is perhaps its ectodermal origin. As a result of this origin it is lined throughout its entire extent by a layer of chitin, which is destitute of pores, and must be regarded as exceedingly impervious. Can it be possible, then, that the "midgut" is, as so many of the authors who have written on the subject have supposed, an organ of absorption? Can we

imagine a structure whose function is to absorb nutrition, lined throughout on the surface which is in contact with the material to be absorbed by a highly impervious layer of chitin?

I was struck with this obvious discrepancy between the structure and the supposed function of the "midgut" early in my studies, and endeavored to test the accuracy of the supposition as to its function. These tests were conducted upon adult individuals of *Armadillidium*, this form presenting, for the purpose I had in view, what I supposed was an advantage over the other genera. This was the occurrence in the epithelial cells of adult individuals of numerous yellowish granules, as I have already stated; it seemed probable, on the assumption that the "midgut" was absorptive, that these granules might be products of assimilation stored up in the cells of well-fed specimens. The fact, however, that in many specimens kept under most favorable conditions for obtaining a plentiful supply of nutrition, the granules did not occur, opened up a way for doubt as to their being assimilation products, and to determine if this were their significance I caused a number of individuals to fast for varying lengths of time by keeping them in glass vessels, the air of which was kept moist by damp filter paper being attached to the cover of the vessels so that it could not be reached by the animals. Some individuals I allowed to fast for four days, others for seven, others for ten, and finally some for fifteen. In every case I obtained the same result as I had from well-fed specimens; the epithelium of some individuals contained the granules, and that of others none, and when the granules were present they were just as abundant as in the well-fed specimens. I concluded, therefore, that the granules could not be assimilation products, since, if they were, they would surely have been completely used up, or at least greatly reduced, before the expiration of a fifteen days' fast.

Before discussing further their significance, however, I wish to mention the result of feeding experiments, which I also tried. Individuals that had fasted for a few days were allowed to feed on powdered cochineal, which they devoured at first with avidity. As soon as the cochineal began to be passed from the intestine as fæces, the animals were killed and the "midgut"

examined; and although I repeated this experiment many times and found in every case the intestine filled with cochineal, yet never did I find the slightest trace of its absorption by the "midgut." On the other hand in every instance the liver cæca were strongly tinged by the cochineal.

These results show that *the "midgut" of Armadillidium does not possess an absorptive function; it merely serves for the passage of undigested material to the exterior.* Digestion and absorption are both performed by the liver cæca, and apparently by them alone. I do not intend to review here the somewhat voluminous literature on digestion and absorption in the Invertebrates, but will merely point out that Cuénot ('92) has obtained results concerning the process of absorption in the pulmonate Molluscs identical with those I have just described for *Armadillidium*.

A fact interesting in connection with these results is the complete absence of a "midgut" (and rectum) in certain parasitic Isopods, and its great reduction in others. Thus in the Bopyridæ, according to the observations of Kossmann ('81) on *Bopyrina Virbii*, the intestine, behind the point where the liver cæca communicate with it, is very narrow and possesses only an exceedingly fine lumen. On the other hand, the two liver cæca are very large,—in fact they might be described as large pouches, and they communicate with the intestine by such wide mouths that it is proper to speak of the presence of a true midgut in these forms. Kossmann says: "Dass durch den Mitteldarm Nahrung—und wir müssen ja hier an flüssige denken—direkt in den Enddarm treten könne, ohnezunächst die Leberhölräume so gut wie vollständig zu erfüllen, ist offenbar unmöglich." This arrangement seems to me at once comprehensible if the physiological actions of the liver and the intestine are as I have supposed them to be in the terrestrial Isopods. In parasitic forms the amount of fæcal matter is relatively very small, while the amount of material to be absorbed is large. The intestine, not being absorptive but merely serving as a passage for the extrusion of the undigested material, is, in *Bopyrina*, exceedingly reduced, while the digestive and absorptive liver pouches are enlarged.



This degeneration of the "midgut" is carried to its complete extent in the Entoniscidæ, according to the observations of Giard ('78) and Kossmann ('81a), there being no continuation of the digestive tract beyond the point where the liver pouches open into it. Even though these forms are parasitic, it is difficult to understand the complete absence of the intestine if it be absorptive in function, since even in such forms as the Trematodes there is a necessity for a well-developed absorptive intestine. If, however, the intestine is not absorptive, then its disappearance in these cases of extensive parasitism is not remarkable.

What, then, is the explanation of the curious structure of the epithelial cells and of the occurrence of the granules and vacuoles in them? I believe that *all the peculiarities are due to catabolic changes*. I have stated on a previous page my reasons for the belief that the epithelial cells persist throughout the entire life of the animals; and I have shown that the development of the vacuoles, of the granular structure of the protoplasm, and of the supportive fibres progresses with age. I regard these changes, therefore, as associated with the senescence of the cells. The fragmentation of the nucleus, not infrequently seen in fully adult individuals, and, indeed, the abnormal forms which the nuclei assume are very probably due to the accumulation in the cells of catabolic products, and I believe the granules which occur in *Armadillidium* are to be regarded as products of this kind, and that their presence indicates approaching dissolution.

Such an opinion as I have here expressed may seem somewhat at variance with the usual conception of an intestine, but I would point out that in the formation of the "brown body" in the Ectoproctous Bryozoa we have something which may be regarded as to a certain extent analogous. Furthermore, it must be remembered that in the Isopods we have to do with an intestine which is in reality entirely an ectodermal proctodæum; and it would, it seems to me, be not only remarkable to find it assuming the functions of an endodermal gut, but also difficult to explain why it should do so when a well-defined endoderm capable of digestion and absorption is present.



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## EXPLANATION OF PLATE IX.

FIG. 1. Surface view of the epithelium of the "midgut" of an adult *Armadillidium*. The drawing of this and of the other surface views is made with the objective focussed at a high level, so that the cells seem to be distinct from one another. (Zeiss, Obj. C., Oc. 2.)

FIG. 2. Surface view of cell from "midgut" of *Armadillidium*. *ch* = optical section of the layer of chitin as it passes down between adjacent cells forming Carnoy's "membrane, secondaire"; *nm* = nuclear membrane; *nl* = nucleoli. (Leitz, Oil-imm.  $\frac{1}{2}$ .)

FIG. 3. Surface view of "midgut" epithelium from an *Armadillidium* which had fasted for 15 days. This figure shows the abundance of yellowish-green granules and also the arrangement of the nuclei which has called forth the theory as to their non-sexual conjugation. (Zeiss, C. 2.)

FIG. 4. Surface view of two cells from the "midgut" of an *Armadillidium* which had fasted 10 days. Showing occurrence of vacuoles (*v*) and yellowish-green granules. The nuclei also show the clear zone of caryolymph within the membrane.

FIG. 5. Section of two cells from adult *Armadillidium*. *ch* = layer of chitinous cuticle which remains unstained; *ch'* that which stains with iron-lack hæmatoxylin *bm* = basement membrane; *m* = muscle tissue; *N* = nucleus; *nl* = nucleolus; *v* = vacuole; *sf* = supporting fibres, one of which (*sf'*) is shown broken across. (Zeiss, D. 2.)

FIG. 6. Two cells from the "midgut" epithelium of *Idotea robusta*, showing the appearance of intercellular bridges of protoplasm, the existence of two nuclei in what is apparently a single cell and the occurrence of intranuclear vacuoles. (Zeiss, D. 2.)

FIG. 7. Epithelial cell from *Armadillidium*, showing a portion of the nucleus apparently fragmented off. (Zeiss, D. 2.)

FIG. 8. Epithelial cells from *Armadillidium*, showing a nucleus which has undergone extensive fragmentation, some of the fragments having also wandered into an adjoining cell area. (Zeiss, D. 2.)

FIG. 9. Nucleus of "midgut" cell of an *Oniscus* which had just left the brood pouch. (Leitz, Hom. Imm.  $\frac{1}{2}$ .)

FIG. 10. Section of a nucleus of a "midgut" cell from an adult *Oniscus*, showing the nuclear membrane *nm* and numerous nucleoli (*nl*) of various sizes. The chromatin is in the form of innumerable granules, and at the left side of the figure a little of the achromatic network is indicated. (Leitz, Hom. Imm.  $\frac{1}{2}$ .)

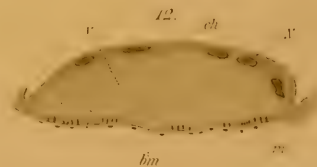
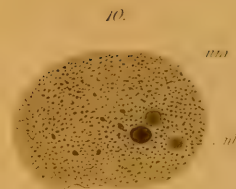
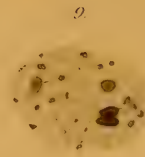
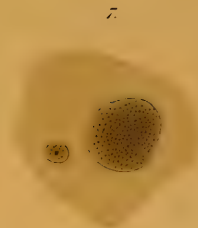
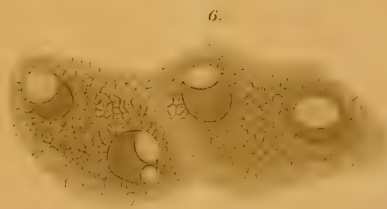
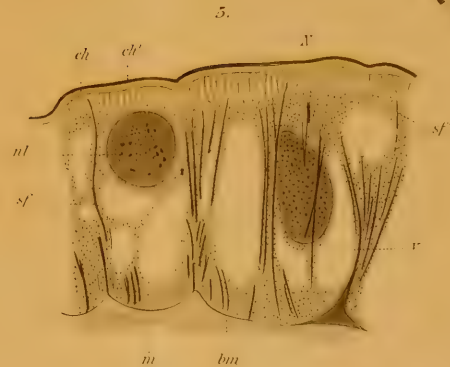
FIG. 11. Section of cell from the "midgut" epithelium of a specimen of *Oniscus* 4 mm. in length. It shows the finely reticulate cytoplasm and the supportive fibres (*sf*) projecting up only a short distance from the basement membrane (*bm*). The clear space around one end of the nucleus is probably an artifact, and the chitinous membrane has been torn away from the surface. (Leitz, Hom. Imm.  $\frac{1}{2}$ .)

FIG. 12. Section through one of the large blister-like vacuoles (*v*) which occur abundantly in the "midgut" of *Armadillidium*. *ch* = chitinous membrane; *bm* = basement membrane; *m* = muscle fibres cut across; *N* = nucleus.











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EXPERIMENTAL STUDIES ON THE DEVELOPMENT OF LIMB-MUSCLES IN AMPHIBIA.

ESTHER F. BYRNES

THE muscles in the fins of elasmobranchs develop from muscle-buds which are formed from the ventral edges of the myotomes. Until very recently this method of formation of limb-muscles was supposed to occur not only in the different groups of fishes, where muscle-buds are best developed, but also in the higher groups of vertebrates.

Muscle-buds have been shown to occur among fishes, in the teleosts as well as in the elasmobranchs; and among the higher vertebrates strong evidence of the presence of muscle-buds in the Lacertilia<sup>1</sup> has been given by Van Bemmelen<sup>2</sup> and Mollier.<sup>3</sup> In the remaining groups, however, the evidence in regard to the presence of muscle-buds is far from being conclusive.

Another method of formation of limb-muscles has been described by Paterson,<sup>4</sup> and still more recently by Harrison.<sup>5</sup>

<sup>1</sup> According to Corning, the ventral myotome-processes in the anterior limb-region in the lizard do not go into the limbs as muscle-buds. Corning believes that the so-called "muscle-buds" are only those ventral myotome-processes which give rise to the muscles of the tongue.

<sup>2</sup> *Anat. Anz.*, Bd. 4, 1889.

<sup>4</sup> *Quar. Journ. Micr. Sci.*, Vol. xxviii, 1887.

<sup>3</sup> *Anat. Hefte*, 1895.

<sup>5</sup> *Archiv. f. micr. Anat.*, Bd. 46, 3, 1895.

In his work on the fate of the muscle-plate in the chick, Paterson has been unable to find muscle-buds or structures homologous to them taking part in the formation of the limbs. According to his interpretation, the limbs are derived wholly from the somatopleure.

Harrison, in a recent contribution to the literature on the origin of the fin-muscles in teleosts, has conclusively shown that in the salmon neither muscle-buds nor myotome-derivatives are *essential* to the development of fin-muscles. In support of this view, Harrison finds that, while most of the fins of the salmon follow the rule in deriving their muscles directly from the myotomes, in the form of muscle-buds, the median and pectoral fins furnish exceptions to the rule. According to Harrison, the muscles of the middle portion of the dorsal fin of the salmon are developed from muscle-buds, while at the anterior end of the same fin all of the characteristic structures of the fin are developed out of a mass of mesenchyme cells, which are entirely independent of the myotomes. In the pectoral fins modification has gone still further. The ventral myotome-processes arising from the ventral edges of the myotomes in the region of the pectoral fins take no part whatever in the formation of pectoral fin-muscles, but, after becoming detached from the myotomes, unite with one another to form the coracohyoid muscle. All the characteristic structures of the pectoral fins, muscles as well as cartilage and connective tissue, develop directly from the somatopleure by the differentiation of mesenchyme-like cells. These two views regarding the origin of limb-muscles can no longer be regarded as incompatible when both modes of muscle formation occur, not only in different fins in the same individual fish, but even side by side in the same fin.

The starting-point in the more recent researches on amphibian limb-muscles has been the hypothesis that the muscles of the limbs are developed from myotome-derivatives which are homologous to the muscle-buds in the fins of fishes. According to Goette's<sup>1</sup> account, the limb-muscles of *Bombinator* develop from cells derived from the outer layer of the muscle-plate.

<sup>1</sup> "Die Entwicklungsgeschichte der Unke," 1875.



Kaestner<sup>1</sup> has failed to show that the myotomes take any part directly in the formation of the limbs in the Anura. Nevertheless, if I have understood him rightly, he still believes that the limb-muscles are derived directly from the myotomes, although he has not seen the process, owing, as he believes, to the very early period at which it takes place.

Still more recently, H. H. Field<sup>2</sup> described myotome-processes taking part in the formation of the muscles of the anterior limbs in the Urodela (*Amblystoma*, *Triton*). He has also extended his observations to several of the Anura (*Rana*, *Bufo*) with similar results. It is unnecessary to discuss in detail the origin of limb-muscles in the higher groups of vertebrates, as this has been very fully treated elsewhere in the literature on the subject. It is sufficient to say that, with a single exception, in all the higher vertebrates in which true muscle-buds have not been found masses of proliferated cells, which are regarded as homologous to muscle-buds, have been described. It was with the hope of definitely determining, if possible, the source of the muscles in the limbs of some of the Amphibia that the present investigation was undertaken, at the suggestion of Dr. R. G. Harrison.

I gladly take this opportunity of acknowledging my many obligations to Dr. Harrison for the friendly interest with which he has followed my work, and for having placed at my disposal much of his own prepared material. I also take pleasure in thanking Prof. T. H. Morgan, in whose laboratory most of the work was done, for his kind criticism. My thanks are also due to Prof. C. O. Whitman, of the Marine Biological Laboratory at Wood's Holl, Mass., where the work was completed.

### *Material.*

The material used in the present investigation consisted of Urodela, *Amblystoma punctatum*, and several species of *Triton*, and of Anura, *Rana sylvatica*, *Rana palustris*, and *Bufo americanus*, collected in the vicinity of Bryn Mawr, Penn.

<sup>1</sup> *Archiv f. Anat. u. Phys. (Anat. Abth.)*, Hefte 5 und 6, 1893.

<sup>2</sup> *Anat. Anz.*, Bd. 9, 23, 1894.

*Methods.*

Most of the embryos were killed in a saturated solution of corrosive sublimate to which five per cent acetic acid had been added. The embryos were subsequently sectioned and stained on the slide with Delafield's haematoxylin, followed by a wash of picric alcohol. This method, when used on amphibian embryos, gives a very sharp differentiation of muscle-fibers, staining them a bright yellow color, while the chromatin in the nuclei remains a deep purple; the protoplasmic network remains faintly stained with the haematoxylin. Other double stains were used to detect the presence of muscle-fibrils, but they gave no better results than the method already described.

I. NORMAL DEVELOPMENT OF THE MYOTOME-DERIVATIVES,  
ANTERIOR AND POSTERIOR LIMBS IN THE URODELA (AMBLYSTOMA AND TRITON).

Since the limb-muscles are derived directly from the myotomes in some of the lower vertebrates, and a similar process is believed to take place in the higher vertebrates, the development of the limb-muscles in higher forms must be studied in connection with the fate of the myotome-derivatives. Maurer<sup>1</sup> has already described the formation of myotome-processes in Amblystoma (Siredon) and has given a detailed account of their subsequent development into the muscles of the body-wall. He has not, however, considered the relation of the myotome-processes to the limbs. As my attention has been directed chiefly to the earlier stages in the formation of myotome-processes and to their relation to the limbs, I shall, for the sake of completeness, give a brief account of the ventral myotome-processes up to the time when they first begin to develop muscle-fibrils.

In Amblystoma all the myotomes of the trunk-region, excepting the first and second, give rise to ventral myotome-processes which are alike in structure. The myotome-processes develop first in the anterior part of the body, where they appear

<sup>1</sup> *Morph. Jahrb.*, Bd. 18, Heft 2, 1892.

as masses of embryonic cells densely packed with yolk-granules, and form projections from the ventral, outer borders of the myotomes (Pl. X, Fig. 1).

Even in their earlier stages the myotome-processes appear as distinct diverticula whose cavities are continuations of the myocoel. As the myotome-processes elongate and the yolk-granules are gradually absorbed, the cells of the rudimentary ventral processes become arranged in a single row along the walls of the myocoel, as shown in Pl. X, Fig. 2. During the ventral growth of the myotome-processes, the cells lose their rounded, embryonic appearance and become elongated dorso-ventrally in the direction of growth. So pronounced is the dorso-ventral elongation of the nuclei of these cells that their characteristic shape often serves as a means of identifying the cells of the myotome-processes, even when they are closely crowded by other structures, as in the region of the anterior limbs (Pl. X, Fig. 4). As the myotome-processes elongate, the myocoel becomes greatly reduced, and finally wholly disappears, leaving double strands of nuclei in place of the thick-walled diverticula of the younger stage (Pl. X, Figs. 6, 7).

While these changes are going on the myotome-processes in the fourth and fifth trunk-segments become separated from their respective myotomes by the developing pronephros. Throughout the length of the body the myotome-processes on each side, including those that have lost their connection with the myotomes, become united into a thin lateral sheet of muscle ("the primary abdominal muscle"). The primary abdominal muscles taper anteriorly into narrow strands which pass to the ventral side of the body, where they become attached to the hyoid cartilage. This anterior, ventral portion of the primary abdominal muscle becomes the sterno-hyoid muscle. In its mode of origin it closely resembles the coraco-hyoid muscle of the teleosts.<sup>1</sup> Beyond this stage I have not followed the ventral

<sup>1</sup> Harrison has shown that in the salmon the myotome-processes which are very early constricted off from the ventral edges of the anterior myotomes in the region of the pectoral fin do not go into the fin as muscle-buds, but unite with each other to form the coraco-hyoid muscle. Corning formerly described these detached myotome-processes in the pectoral fin-region as muscle-buds. Later he confirmed Harrison's account.

myotome-processes, as their subsequent fate has no bearing on the question of the origin of the limb-muscles. The details of their later development are given in Maurer's paper on Siredon.

Simultaneously with the early development of the myotome-processes the anterior limbs begin to develop. They originate as somatopleuric thickenings formed by an aggregation of mesenchyme-like cells around a slight fold of the epithelium bordering the coelom (Pl. X, Fig. 1). H. H. Field has already called attention to this fold and its relation to the rudimentary limb.

The anterior limb-rudiments develop some distance *below* the level of the myotomes; hence, when the myotome-processes elongate ventrally they project toward the limbs. During the very early stages of development all the cells are so crowded with yolk-granules that the line of demarcation between the myotome-processes and the rudimentary limbs is often obscured. In favorable cases, however, a line of pigment bordering the myocoel outlines the extent of the process so that it can be followed (Pl. X, Figs. 3, 4). The apparent fusion of the myotome-process with the rudimentary limb-mass can often be explained as due to the fact that the myotome-processes are cut obliquely. Such sections often show no distinct space between the myotome-process and the limbs. The true relations between the myotome-processes and the anterior limb-rudiments in *Amblystoma* are further obscured by the early appearance of the second pronephric funnel in the region of the limbs (Pl. X, Fig. 3). As the pronephric tubules increase in length they elongate at right angles to the direction of growth of the myotome-processes, and consequently push the myotome-process over against the somatopleuric thickening of the limb. The close proximity of the two structures seems to be due mainly to mechanical causes; *i.e.*, to the pressure exerted on the myotome-process by the elongating tubules. When the tubules become convoluted, later, they force their way in between the rudimentary limb and the myotomes, so that the connection between the myotomes and the ventral myotome-processes is broken (Pl. X, Figs. 6, 7). Maurer<sup>1</sup> has already shown these relations in the Urodela.

<sup>1</sup> *Morph. Jahrb.*, Bd. 18, 1, 1891. Fig. 7, Pl. VI.



It is the ventral myotome-process in the region of the second pronephric funnel which H. H. Field has called "Unwirbelknospe," and from which he derives the pronephric capsule and the limb-muscles in *Amblystoma*. Posterior to the region of the pronephric funnels, the myotome-processes permanently retain their connection with their respective myotomes, and pass directly by the inner side of the limb without any appearance of fusion with it (Pl. X, Figs. 4, 5).

These relations are even more clearly seen in *Triton* than in *Amblystoma*, for, while the body of *Triton* is relatively wider, it has a shorter dorso-ventral axis. Hence, as the ventral edges of the myotomes are nearly on a level with the anterior limb-rudiment, when the ventral myotome-processes are formed, instead of pointing toward the limb-rudiment, as in *Amblystoma*, the myotome-processes pass directly by its inner side (Pl. X, Fig. 11). In both *Triton* and *Amblystoma* the independence of the myotome-processes and the anterior limbs becomes more marked in older larvae after the limbs have begun to project slightly from the sides of the body.

The yolk can be largely removed from young *Amblystoma* larvae so that whole preparations can be mounted in glycerine or balsam and thus be made perfectly transparent. In older embryos the myotome-processes are so closely applied to the walls of the coelom that it is often difficult to remove the yolk without completely tearing away the myotome-processes or breaking them. When the myotome-processes are removed, the anterior limb-rudiments *always remain intact* as a uniform thickening of the somatopleure in which there is no evidence of any structure comparable with muscle-buds as they appear in fishes. This tearing away of the myotome-processes without disturbing the limb-rudiments seems to indicate an independence of the myotome-processes and the limbs. Pl. X, Fig. 17, is intended to show these relations in the anterior limb-region of a young *Amblystoma* larva.

When the ventral myotome-processes (*i.e.*, the "primary abdominal muscles") and the limb-rudiments in the anterior part of the body of *Amblystoma* are well developed, the myotome-processes and the limbs in the posterior part of the body



are still in a very rudimentary condition. In the immediate region of the posterior limbs the myotome-processes are represented only by a few embryonic cells at the ventral outer corner of the myotome (Pl. X, Fig. 13).

Fig. 15 represents a section through the posterior part of the body of a young Triton larva, some distance in front of the posterior limb. The myotome-process is represented by only a few cells at the ventral outer edge of the myotome. These cells are the forerunners of the primary abdominal muscle, with which they are connected in a more anterior section. One of the cells is shown in the act of dividing. Such cases of division are of frequent occurrence at the ventral edges of the myotomes in the posterior part of the body and are incidental to the growth of the abdominal muscle.

Fig. 14 shows a section taken through the posterior limb-rudiment of a Triton embryo. The conditions shown at the ventral edge of the myotome in front of the limb (Fig. 15) differ in no way from those in the limb-region itself, except that in front of the limb the myotome-processes have begun to grow ventrally. On account of the close proximity of the posterior limb to the ventral edge of the myotomes, there is sometimes an apparent connection between the two structures. Nevertheless, I believe this is only a coincidence, for, after having examined a large number of sections through the posterior part of the body, including the limb-region, I have been unable to find any special localized proliferation of cells from the myotomes to the limbs. Moreover, the number of dividing cells at the ventral edge of the myotomes in the limb-region is relatively very small. I cannot believe that in *Amblystoma* there is any ground for homologizing these single cells with muscle-buds when typical muscle-buds are absent.

Among the higher groups of vertebrates, where muscle-buds, such as are found in the fishes, have not been shown to exist, the cells that are proliferated from the ventral edges of the myotomes have been very generally homologized with true muscle-buds. But the evidence given in these cases is not wholly convincing that the cells proliferated from the myotomes are really homologous to muscle-buds, or even that they give

rise only to muscle tissue. They are to be regarded rather as the "formative-tissue"<sup>1</sup> cells of Goette and Ziegler. In the elasmobranchs, where "muscle-buds" are best developed, Ziegler has shown that cell-proliferation occurs independently of the formation of muscle-buds and in addition to it.

The first indication of the posterior limbs is seen in a thickening of the somatopleure at the extreme posterior limit of the coelom. The limbs arise ventral to the myotomes and appear first as an aggregation of a few mesenchyme cells lying directly beneath the thickened ectoderm (Pl. X, Fig. 13). By the time the posterior limbs first begin to make their appearance the yolk-granules have been almost wholly absorbed from the mesoderm, leaving the large nuclei lying freely suspended at the nodal points of a protoplasmic network. In this network, which is almost colorless even in stained sections, the nuclear divisions can be easily followed. Since cell migration is of widespread occurrence in the formation of embryonic organs, evidence based upon nuclear division alone cannot be regarded as entirely conclusive in determining the original sources of the cells that go to form any given tissue. Nevertheless, whatever evidence can be deduced from karyokinesis points unmistakably to the somatopleure as the region of growth in the formation of the posterior limbs. This explanation is rendered all the more probable by the frequent proliferation of cells from the endothelium bordering the coelom directly in the limb-region.

Comparing the total number of cases of mitosis at the ventral edges of the myotomes in the limb-region of eleven embryos with the total number of cases in the limb-rudiment, we find that there are 24 dividing nuclei at the edges of the myotomes to 242 in the somatopleure of the limb; or 1 in the myotome to every 10 in the somatopleure.

When we consider that numerous cases of mitosis are also seen at the ventral edges of the myotomes throughout the length of the body and in the tail, the fact that isolated cases of cell division occur at the edges of the myotomes in the limb-

<sup>1</sup> I have used "formative tissue" in Ziegler's sense as referring to cells that are physiologically undifferentiated.

regions can have little weight in establishing the dependence of the limb-muscles on the myotomes.

## II. NORMAL DEVELOPMENT OF THE MYOTOME-DERIVATIVES, ANTERIOR AND POSTERIOR LIMBS IN THE ANURA (RANA AND BUFO).

What has been said of the relation of the myotome-processes to the limbs in *Amblystoma* applies also in a general way to *Rana* and to *Bufo*. The *Anura* differ, however, from the *Urodela* in the structure of the ventral myotome-processes and in the early separation of the myotome-processes from the myotomes. Another point of difference between the two forms and one which has been taken advantage of in experimenting on *Rana* consists in the increased distance between the ventral edges of the myotomes and the limbs (Pl. X, Fig. 18).

Kaestner<sup>1</sup> and Maurer<sup>2</sup> have already given a detailed account of the development of the muscles of the body-wall in the *Anura*, and Kaestner has attempted to show the relations of the myotomes to the limbs. Inasmuch, however, as the present account of the muscles of the limbs differs from any that has been given for *Rana*, I shall briefly review the early development of the myotome-processes during the time that they are in closest contact with the limbs.

In *Rana* the myotomes give rise to short ventral processes which become constricted from the myotomes very early. Unlike the ventral myotome-processes of *Amblystoma* and *Triton*, the ventral myotome-processes of *Rana* contain no myocoel, but appear as solid outgrowths from the ventral edges of the muscle-plates (Pl. X, Fig. 18). The ventral processes of the anurans are first formed from the myotomes in the anterior part of the body. Soon after their formation they are constricted from the myotomes and begin to move toward the ventral body-wall. Later, the more posterior myotomes give rise to ventral processes which likewise become separated from the myotomes in the order of their formation. Throughout

<sup>1</sup> *Archiv f. Anat. u. Phys. (Anat. Abth.)*, Hefte 5 und 6, 1893.

<sup>2</sup> *Morph. Jahrb.*, Bd. 22, Heft 2, 1894.

the length of the body these detached ventral myotome-processes participate in the formation of continuous lateral bands of muscle, which lie one on each side of the body, and which, as Maurer has shown, give rise to the abdominal muscles.

In the tail-region the abdominal muscle-rudiment retains its connection with the myotomes (Pl. X, Fig. 12). It tapers anteriorly to a very delicate strand of muscle, which becomes attached to the posterior edge of the hyoid cartilage just above the heart. Further than this I have not followed the ventral myotome-processes. A full account of their later development has been given by Kaestner and by Maurer. The above account covers the period of growth when, if there had been any connection between the myotomes and the limbs, it would have been evident.

The anterior limbs of *Rana* first appear as a thickening of the somatopleure just behind the gills. I have been unable to trace any connection between the anterior limbs and the myotomes in *Rana*. The two structures are so far separated from each other, not only as regards their actual position, but also as regards the time of their formation, that even if any intimate connection does exist between them it is impossible to follow it in normal embryos.

According to Goette's account, both the anterior and posterior limbs of *Bombinator* derive their muscles from the outer cells of the segmental plate. Jordan in his account<sup>1</sup> of the development of the anterior extremity of the *Anura* accepts the account already given by Goette, and treats only of the later stages of development after the differentiation of tissue has already begun.

The relations between the myotomes and the limbs in the frog can be much more easily studied in the posterior than in the anterior limb-region. By the time the posterior limb-rudiments first begin to make their appearance the ventral myotome-processes in the posterior limb-region have just been constricted off from the myotomes, and have come to lie immediately ventral to them (Pl. X, Fig. 18). The rudiments of the posterior limbs first appear at the extreme posterior limit of

<sup>1</sup> "Die Entwicklung der vorderen Extremität der Anuren-Batrachier."



the coelomic cavity as aggregations of a few of the mesenchyme-like cells of the somatopleure. In the region of this mesodermal thickening the ectoderm also becomes conspicuously thickened. Kaestner has already pointed out these relations in the development of the posterior limbs of *Rana*.

After leaving its posterior attachment to the eleventh myotome, the abdominal muscle-rudiment (the ventral myotome-processes) skirts the anterior limit of the posterior limb, and as it passes forward and ventrally presses so close to the limb that it is often difficult to detect any boundary between the cells of the two structures (Pl. X, Fig. 12). Cross-sections through the posterior limb-region of *Rana* show the abdominal muscle elongated in the direction of the limb and having at times the appearance of a muscle-bud with a well-defined ventral boundary. Longitudinal sections, however (Pl. X, Fig. 12), show that this appearance is due only to the sectioning of an oblique structure, and that there are in reality no bud-like structures present. Directly in front of the limb-rudiment the abdominal muscle emerges at a level below the limb.

The youngest larvae figured by Kaestner<sup>1</sup> are much too far advanced to show the closest connection between the posterior abdominal muscle-rudiment and the earliest rudiment of the posterior limbs. Pl. X, Figs. 12 and 18, show the normal relations between the myotome, the posterior abdominal muscle, and the limb in a very young frog embryo in which the limbs are just beginning to develop. The only possible connection between the myotomes and the limb-rudiments in *Rana* is an indirect one through the abdominal muscle, which at a very early period comes into close contact with the limbs. But even here the connection between the limbs and the abdominal muscle is only apparent, as I hope to show in the chapter on experiments on *Rana*.

Kaestner has been unable to demonstrate an ingrowth of any myotome-derivative into the limb-rudiment in *Rana*. He says: "So scheinen unsere Untersuchungen zu einem Resultat geführt zu haben, welches im Widerspruch steht mit denen, die bisher an allen übrigen Wirbelthier-Klassen gewonnen

<sup>1</sup> *Archiv f. Anat. u. Phys.*, Hefte 5 und 6, 1893.



worden sind. Wir sahen bei Froschlarven aus der undifferenzierten Extremitätenanlage sowohl Knorpel als auch Muskulatur hervorgehen, scheinbar ohne Betheiligung der Myotome. Aber dies auch nur scheinbar, denn vorausgesetzt, dass die Myotome der Froschlarven ebenso wie die der übrigen Wirbelthier Klassen die Grundlage der Extremitätenmuskulatur abgeben, so muss bei den frühesten Stadien von Froschlarven, die ich bisher beschrieben . . . jener Vorgang längst abgeschlossen sein." My own *observations* agree with Kaestner's, but his assumption that the limb-muscles must be derived from the myotomes is, I think, not supported by the facts of development as shown by experimental study.

From the study of normal amphibian embryos, urodeles, and anurans, I was led to conclude that the limbs are wholly somatopleuric in origin, that the myotome-processes take no part in the formation of the limbs, but give rise exclusively to the musculature of the body-wall, and that there is no specialized proliferation of cells from the myotomes in the limb-region. I determined to further test the somatopleuric origin of the limb-muscles by experiment. The experiments were made in the spring of 1895, on young tadpoles (*Rana sylvatica*), and on young Amblystomas (*Amblystoma punctatum*).

### III. EXPERIMENTS ON AMBLYSTOMA AND RANA.

The method of experiment was as follows: Very young embryos in which neither the myotome-processes nor the limb-rudiments had begun to develop, were cut from their capsules and placed in a watch crystal on filter paper moistened with water. Then with a hot needle the ventral halves of the myotomes of the posterior limb-region on the right side of the body were destroyed. A few similar experiments were made with cold needles. These, however, gave no definite results, owing to complete regeneration, and the use of cold needles was abandoned. All the operations were performed under a dissecting microscope. Since in Amblystoma the posterior limbs arise in the twentieth segment, the attempt was made to destroy the ventral edges of the myotomes from the sixteenth to the

twentieth, inclusive. The posterior limbs in the frog tadpoles develop in the eleventh segments. Here the injury extended from about the sixth trunk-segment to the tail. After the operation the embryos were transferred to large shallow dishes of water. Care was taken to change the water frequently within the first few hours after the operation, until the wounds had entirely healed over. After that no further precautions were taken. Most of the embryos survived the injury and were kept in the laboratory in an apparently healthy condition for periods varying from one to eight weeks.

An effort was made to restrict the injury as far as possible to the myotomes, leaving the somatopleure of the limb-region uninjured. I hoped, by completely separating the limbs from the myotomes, to be able to test the power of independent growth of the limbs when all connections with myotome-derivatives had been destroyed. In *Amblystoma* the posterior limb-rudiments arise so close to the ventral edges of the myotomes that it is extremely difficult to destroy the ventral portion of the myotomes without involving the somatopleure of the limb-region more or less in the injury. On this account *Amblystoma* is not a very favorable object on which to study the origin of limb-muscles by the experimental method.

Notwithstanding the distortion that the right side of the body of *Amblystoma* usually undergoes in consequence of the incidental injury to the somatopleure, the right limb often reaches a surprising development, very little, if at all, inferior to the limb on the normal (left) side of the body. Pl. XII, Fig. 37, represents a section through the anterior part of the posterior limb of an *Amblystoma* embryo killed four weeks after injury. The right myotomes have not regenerated their own tissue perceptibly, nor have they given rise to the abdominal muscle-rudiment. In spite of the injury to the myotomes, the limb has reached a surprising development. The limb on the injured (right) side of the body is in every way similar to the one on the normal (left) side, not only in general size, but also in the appearance of its component cells.

Pl. XII, Figs. 38 and 39, show the injured and uninjured sides, respectively, of an embryo, in the posterior limb-region,

six weeks after injury. Fig. 39 represents the posterior limb-region on the uninjured (left) side of the body. The myotome on the right side of the section has been almost wholly destroyed; the right side of the body has so contracted as to rotate the dorsal fin through an angle of nearly ninety degrees. Fig. 38 represents the posterior limb-region on the injured (right) side of the same embryo. The myotome, though not wholly destroyed, is *greatly reduced* in size, only a few muscle-cells remaining. The limb-rudiment has reached a conspicuous development, and is but little inferior to the one on the normal side. The slight reduction in size of the right limb is evidently due to some injury that resulted to the somatopleure of the limb-region when the myotomes were destroyed. Evidence of this is seen in the very general distortion of the whole right side of the section.

Fig. 40 shows the right limb of an *Amblystoma* embryo killed forty-four days (six weeks and two days) after injury. In the limb on the normal (left) side of the body cartilage and muscle-fibrils are already present. In the limb on the injured (right) side the cartilaginous areas are distinctly marked out, although the cartilage is not so well developed as in the limb on the uninjured side. Differential stains fail to show the presence of muscle-fibrils in the right limb, although the *muscle areas are distinctly marked out* around the central core of cartilage. Moreover, they correspond in every respect to the muscle-areas in the limb on the normal (left) side of the body. The entire mass of the right limb is less than that of the left limb, but the reduction in size, as well as the lesser degree of differentiation, in the right limb is evidently due to a general retardation of growth resulting from the injury to the right side of the body, and not to any difference in the *kinds* of tissue present. The permanently reduced size of the myotome bears witness to the extent of the original injury, and leaves little doubt that the region of growth of the myotome was completely destroyed. Even if the limbs were dependent on the myotomes for their muscle-tissue, it seems scarcely conceivable that the myotomes could, in the mutilated condition shown in the figures, contribute cells in sufficient numbers to enable the

limb on the side of the injury to keep pace with the limb on the uninjured side of the embryo *without first regenerating themselves*.

A few preliminary experiments were made on the tadpoles of *Rana sylvatica*, and these showed that the Anura offer much more favorable conditions for the experimental study of the limb-tissues than do the Urodela, as has already been stated. This is owing to the greater distance between the myotomes and the limbs in the Anura, — a condition which makes it possible to destroy the myotomes without necessarily involving the somatopleure of the limb-region in the injury.

On the 10th of April, 1895, eighty embryos of *Rana palustris* (7 mm. to 8 mm. in length) were taken from their capsules, and the ventral halves of the myotomes in the posterior limb-region were destroyed. On the 11th of April similar operations were made on forty more embryos from the same set of eggs. In all cases the operation was performed on the right side of the body. All these embryos (one hundred and twenty in number) survived the operation and were kept in the laboratory in an apparently healthy condition for periods varying from two days to six weeks. Some of these embryos showed the effects of the injury by a very perceptible shortening of the dorso-ventral axis of the right side of the body and by a bending of the tail sideways through an angle of ninety degrees. From the 12th to the 23d of April some of these one hundred and twenty tadpoles were killed daily. After that, a few were killed at intervals of several days until the 4th of June, when all the remaining tadpoles of the set were killed. Normal embryos were also preserved as a check to the injured series.

Many of the injured embryos have been disregarded as giving inconclusive results. None of the discarded embryos, however, furnish any evidence against the somatopleuric origin of the limb-muscles. Many of them show only the normal relations, in the posterior limb-region, between the myotomes, the abdominal muscles, and the limbs. In some of the earlier experiments the injury was confined to the extreme posterior part of the body. Many embryos, thus injured, were kept for several



days and were then killed and sectioned. They show that the trunk-segments have entirely escaped injury, although the myotomes in the proximal part of the tail have often been reduced to one-half their original length and the whole right side of the tail has become greatly contracted. This apparent migration of the injury seems to be due to the gradual growth of the tail. As the tail lengthens, myotomes that in younger embryos seemed to belong to the trunk appear later as anterior tail-segments.

Only those cases have been considered and figured in the present paper which show marked traces of the early injury to the myotomes and in which only a very slight injury, if any, has been sustained by the somatopleure of the limb-region. Normal frog embryos killed on the 10th and 11th of April and used as a check to injured embryos show that at the time of the operation neither the primary abdominal muscle-rudiments nor the posterior limbs had begun to develop. Therefore, no myotome-derivatives could at this time have been given to the limbs.

Pl. XI, Fig. 19, serves as a control for the injured embryos. On the right side of the section the myotome is reduced to almost one-third of its length, and the abdominal muscle-rudiment on the right side has been completely destroyed. The limb-rudiment is uninjured and is almost as well developed as the limb on the normal side of the embryo.

Sections through the posterior limb-region of an embryo killed three days after injury show that the myotomes on the side of the injury have been greatly reduced throughout the limb-region. The abdominal muscle-rudiment is wanting in corresponding sections, and there is little evidence of any attempt at regeneration. The limb-rudiment, which consists of only a slight aggregation of mesenchyme-like cells, is present and normal. Another embryo killed three days after injury shows a double abdominal muscle-rudiment on the side of the injury. A small mass of cells constricted from the myotome lies a little below and to the inner side of the myotome. At the ventral, outer edge of the myotome there is a second mass of cells similar to the first and occupying the normal position of the primary abdominal muscle-rudiment.



Sections of an embryo killed four days after injury show that the myotomes have not been very greatly reduced, although the extreme ventral edges, together with the abdominal muscle-rudiment, have been destroyed. The somatopleuric thickenings of the limbs are equally developed on both sides of the body.

Pl. XI, Figs. 19 and 20, represent sections taken through the posterior and anterior parts of the posterior limbs, respectively, of an embryo killed four days after injury. Fig. 19 shows the primary abdominal muscle on the normal side of the embryo, still in contact with the myotome. In Fig. 20 the primary abdominal muscle has become constricted off from the myotome on the normal side, and is approaching the extreme anterior limit of the limb-rudiment. There is as yet no evidence of regeneration of the abdominal muscle on the part of the injured myotomes on the right side.

An embryo killed seven days after injury shows that the myotomes of the posterior limb-region have been reduced to one-third their normal length. Notwithstanding the injury to the myotomes, the abdominal muscle has begun to regenerate. Evidence of this regeneration is seen in the general tendency of the mesenchyme-like cells between the myotomes and the limb to elongate dorso-ventrally, and to fall into line along the path of the developing abdominal muscle. The right limb, though slightly smaller than the left, is well developed. The difference in the size of the two limbs in this embryo is due to the fact that the somatopleure, as well as the myotomes, has been injured. This is evident from the abnormal arrangement of the blood vessels on the side of the injury. Another embryo killed seven days after injury shows the right myotomes greatly reduced throughout the whole extent of the limb-region. On the side of the injury the abdominal muscle is beginning to regenerate. Both the posterior limb-rudiments are present and normal. Still another embryo killed seven days after injury shows the ventral halves of the myotomes in the right posterior limb-region completely destroyed. Near the limb the right abdominal muscle has begun to regenerate. Both the limb-rudiments appear normal.

An embryo killed eight days after injury shows the right myotomes reduced to one-half their original length. In the posterior part of the body, where the abdominal muscle and the myotomes remain permanently connected, the myotome has begun to regenerate dorso-ventrally. In front of this place of connection, however, the myotomes give little evidence of any dorso-ventral growth. The regenerating abdominal muscle has already developed well-marked muscle-fibrils, and both the limbs are normal.

An embryo of an unknown species of frog killed nine days after injury shows a marked reduction in the size of the myotomes; it also shows a normal development of the limbs. Pl. XI, Fig. 41 *a*, shows a section through the posterior part of the posterior limb-rudiment of this embryo. On the normal (left) side of the body the abdominal muscle has not, as yet, been constricted from the myotome. The limb-rudiment is well developed. On the injured (right) side, the section shows the right myotome almost wholly destroyed, and with it also the abdominal muscle-rudiment. The injury has also involved the medullary tube, no trace of which remains in the limb-region. Notwithstanding the extent of the injuries, the right limb-rudiment is apparently normal. Fig. 41 *b* represents a section through the anterior limit of the posterior limb of the same embryo. On the uninjured side of the body the abdominal muscle-rudiment has not as yet come in contact with the limb. On the injured side the myotome is still greatly reduced, but shows a tendency to regenerate toward the limb-region. Although the myotome is not wholly destroyed on the right side of the embryo, it is so mutilated that it is scarcely conceivable that the few remaining muscle-cells could contribute cells to the limb, even if they did so normally. The limb-rudiments are, however, equally large on both sides of the body, as is shown in Fig. 44, which represents a camera drawing of the limb-region indicated in Fig. 41 *a*.

Sections of an embryo killed eleven days after injury show that the outline of the body has not been distorted. The injured myotomes on the right side of the body extend ventrally only to the level of the notochord. The abdominal

muscle has regenerated so as to occupy an almost normal position at the anterior limit of the limb; it does not, however, extend throughout the entire limb-region, as is the case in normal embryos. The limb-rudiments are well developed and normal. In another embryo killed eleven days after injury the myotomes have been reduced to almost one-half their original length. The abdominal muscle-rudiment has regenerated, but is present in the limb-region only at the extreme anterior limit of the right limb. Both limb-rudiments are normal.

An embryo killed thirteen days after injury shows that the myotomes of the posterior limb-region on the right side of the body have been reduced to one-third their original length. The right abdominal muscle, though present, is reduced in size and shortened. Notwithstanding the abnormal conditions in the myotomes and the abdominal muscles, the limbs are well developed and normal.

Figs. 21-25 represent sections through an embryo killed twenty-six days after injury. Owing to the distortion that has resulted from the mutilation, the sections do not show corresponding regions of the right and left limbs. The limbs extend, however, over an equal number of cross-sections, and are apparently in every way similar. Fig. 21 is taken through the mid-region of the limb on the injured (right) side of the body. The myotome is reduced and the abdominal muscle-rudiment is wanting. The limb is well developed and normal. Fig. 22 shows a more anterior section through the most anterior part of the right limb, which is represented, in this section, by only a few cells in the dorsal wall of the coelom. The myotome has begun to regenerate ventrally, but has not as yet reached the level of the limb.

Fig. 23 is taken directly in front of the limb-region. It shows the ventral growth of the myotome from which the abdominal muscle is to be, at least in part, regenerated. The relations of the abdominal muscle in still more anterior sections are represented in Figs. 24 and 25. These sections plainly show that the myotomes are still greatly reduced and that their ventral regions of proliferation must have been completely destroyed when the injury was first made. The limbs are,

nevertheless, normal. The right limb is evidently entirely separated from the myotome or any of its derivatives; but it is, notwithstanding its isolation, in every way comparable with the limb on the uninjured (left) side of the embryo. The abdominal muscle has regenerated, *but only in front of the limb.*

An embryo that had been injured on the 4th of April and killed on the 6th of May (thirty-two days after injury) shows but little trace of injury to the myotomes. The right myotomes are slightly shortened, but the relative distances between the myotomes and the limbs on the right and left sides of the body are about the same and normal. A reconstruction of the embryo shows, however, that, *while the primary abdominal muscle is present throughout the whole extent of the limb-region on the normal (left) side, on the injured (right) side the muscle is present only at the anterior limit of the limb, and even there it is reduced to nearly half its normal size.* The limbs are well developed and seemingly normal; they are, moreover, equal in size, and in both limbs the cells that will give rise to muscles are already clearly distinguishable from those that will form the cartilage. As yet, however, the muscle-fibrils have not appeared in either of the rudimentary limbs. Pl. XII, Figs. 46 and 47, show corresponding sections through the right and left limbs of this embryo.

Sections of an embryo killed thirty-three days after injury show that the myotomes have been greatly reduced. The abdominal muscle-rudiment comes in contact with the limb only at its *extreme anterior limit.* Both limbs are equally well developed.

One of the embryos killed thirty-four days after it was injured proved to be of particular interest. Pl. XI, Figs. 29-36, inclusive, show a series of sections taken through the limb-region of this embryo. The myotomes are but slightly shortened. Throughout the limb-region on the right side of the body there is *no indication whatever of the abdominal muscle-rudiment.* Only at the most anterior limit of the limb does the muscle begin to make its appearance (Fig. 36), and then it is represented by only a single muscle-cell in which fibrils are well developed. Still more anteriorly, in front of the limb-region, the right abdominal muscle-rudiment remains



greatly reduced, not only in thickness, but also in length. On the left side of the body the abdominal muscle has reached a very conspicuous development. Although the right limb is apparently free from contact with any of the myotome-derivatives, it has reached a development equal to that of the limb on the normal (left) side of the embryo. The two limbs are in every way comparable, not only in size, but also as regards their regions of differentiation within the limb-bud, as shown in Pl. XII, Fig. 45.

One of the embryos shows the myotomes in the posterior limb-region reduced to one-half their normal length, even after six weeks have elapsed since the operation. A few scattered muscle-cells lie between the lower edge of the injured myotome and the limb-region. Whether some of the original myotome cells have escaped injury or whether those now present have regenerated is not clear from the sections. The injury was an extensive one, however, and must have affected the growth of the limb-rudiment had the limb been dependent on the myotomes either directly or indirectly for its development. The abdominal muscle is present, though somewhat reduced. Directly in front of the limb-region the abdominal muscle is well developed. Both the limbs are normal.

The results of these experiments both on *Amblystoma* and *Rana* confirm the conclusions already reached from the study of normal embryos; *i.e.*, that the muscles of the limbs are developed wholly out of the mesoblastic cells of the somatopleure, the myotome-processes taking no part in the formation of the limb-muscles. First of all, the myotomes after injury remain permanently reduced in size. When the myotomes are greatly shortened there is often a corresponding shortening or contraction of the entire injured side of the body. In the later stages of these embryos there is no evident attempt on the part of the myotomes to regain their normal proportions. Even when the myotomes have been but slightly injured, only the extreme ventral edges being destroyed, there is generally some permanent<sup>1</sup> indication of the injury in a reduction in the size of the muscle-plates.

<sup>1</sup> None of the embryos used in these experiments were kept longer than eight weeks.



Although the myotomes plainly show that they have been reduced by the operation, and the regions from which the myotome-processes develop have been destroyed, a rudiment of the primary abdominal muscle is always present, even though it is often greatly reduced in size. The presence of an abdominal muscle-rudiment, together with a greatly reduced myotome on the side of the injury, might seem to indicate that the primary abdominal muscle-rudiment had formed prior to the time of injury to the myotomes and had possibly escaped being destroyed when the myotomes were injured. Normal embryos killed on the same days that the others of the set were injured and used as a check to the injured series show that the ventral myotome-processes or primary abdominal muscle-rudiments are still in connection with the myotomes, even in the anterior part of the body, where the connection between the myotomes and their ventral processes is earliest lost. In the posterior limb-region of the "check" embryos the abdominal muscle-rudiment is represented by only the extreme ventral edges of the myotomes, which had not begun to be constricted at the time of the operations.

It is not probable that the abdominal muscle-rudiment in corresponding embryos always escaped being destroyed throughout the extent of the injury when the myotomes have been as greatly reduced as shown in Pl. XI, Figs. 24 and 25. I have made several sets of experiments on the tadpoles of *Rana palustris* to see if the primary abdominal muscle really does always regenerate after it has once been destroyed. These experiments consisted in destroying the ventral halves of the myotomes before the primary abdominal muscle had begun to develop. The injury was much more extensive in these experiments (made in the spring of 1896 and of 1897) than it was in the original ones (made in 1895), for it was not confined to the limb-region, although it often included the limb.

The results of the experiments show that when the rudiment of the abdominal muscle is destroyed along with the ventral edges of the myotomes it always regenerates, the process of regeneration beginning within a few days after the injury. I have never found an embryo in which a rudiment of the

abdominal muscle was wholly wanting. There is in nearly all of the injured embryos a dorso-ventral growth from the myotome in the posterior part of the body, where the rudiment of the abdominal muscle and the myotomes retain their connection with each other. This down-growth from the myotome, such as is shown in Pl. XI, Figs. 23, 24, and 27, occurs in connection with the regeneration of the abdominal muscle, and not in connection with the regeneration of the myotome itself. Directly in front of this place of union of the abdominal muscle with the myotome (Figs. 23, 27), there is no corresponding down-growth from any of the myotomes, but the rudimentary muscle is always present some distance below them (Figs. 25, 28).

Since all the myotomes of the same embryo were injured at the same time, I expected to find them all simultaneously undergoing similar regenerative changes, but I have never found this to be true. I am unable at present to satisfactorily explain the regeneration of the abdominal muscle, and can only suggest one of two hypotheses by way of explanation: either the abdominal muscle is formed anew (by the injured myotomes) from a second series of myotome-processes, or it is regenerated independently of the myotomes from an uninjured part of the muscle itself.

On the hypothesis that the abdominal muscle regenerates from a series of down-growths from the injured myotomes, the anterior myotomes would have to regenerate their ventral myotome-processes before the posterior myotomes regenerated theirs, for the position of the regenerated muscle-rudiment in the body-wall is almost invariably *oblique*; the more anterior end being much further ventral to the myotomes than the posterior end of the muscle, which always remains in contact with the myotome from which it was originally derived. This hypothesis could account for the regeneration of the abdominal muscle in the posterior part of the body where the myotome and the muscle-rudiment are connected, but it cannot explain the regeneration of the abdominal muscle in front of this region.

The oblique position of the regenerating muscle and the complete separation of the muscle from the anterior myotomes

are more readily explained by the alternative hypothesis ; *i.e.*, that the abdominal muscle has regenerated from its own tissue in an uninjured part of the body, rather than from the injured myotomes themselves. This suggestion is made probable by the fact that the connection between the *regenerating* abdominal muscle and the *original* abdominal muscle-rudiment is *always unbroken*, the regenerating muscle always passing gradually into the normal muscle, which lies ventrally in a more anterior part of the body. This could hardly be the case if the regenerating muscle were formed by a second series of ventral processes from the myotome.

Although the rudiment of the abdominal muscle regenerates sooner or later, the new muscle is often smaller than the corresponding normal one on the uninjured side of the body. It is also often much nearer the ventral edge of the myotomes than the one on the normal side. These experiments, showing that the abdominal muscle always regenerates, make it extremely probable that in the original experiments the rudiment of the abdominal muscle was actually destroyed, but that it has regenerated and in some cases has reached almost its original size. Although the rapid regeneration of the rudimentary abdominal muscle often brings about normal relations between the muscle and the limb-rudiment, the injury often suffices to check the development of the myotomes and to keep them, as well as the myotome-derivatives, temporarily from coming in contact with the limb-rudiments.

The most striking fact in connection with the injured embryos is that, notwithstanding the permanent reduction in the size of the myotomes and the consequent diminution in the size of the myotome-derivatives, *the limbs are normal*. This normal development of the limbs can only be explained on the ground of independence of the limbs and the myotomes. If the limbs are normally dependent on the myotomes for so large a proportion of their entire mass as is represented by the muscles, *why*, when the source of the muscles has been destroyed, *is there no corresponding diminution in the size of the limbs?* There is no reduction in the limbs corresponding to the reduction in the muscle-structures, except in those cases where the

injury to the myotomes has also involved the somatopleure immediately below them.

In such cases, however, the reduction in the size of the limbs is due to interference with metabolic processes, and is not due to the exclusion from the limbs of masses of cells that are destined to provide them with any given tissue. Evidence that the limbs are not dependent on the myotomes for any such large proportion of their tissues as is represented by the muscles is given in Pl. XII, Figs. 42-47. All these cases, besides others which have not been figured, go to show that the limbs develop alike on both sides of the body, and normally, even though the muscle-structures have been very largely destroyed.

The regions in which the different tissues are going to develop become clearly outlined in the limbs long before differentiation into muscle and cartilage actually begins. The peripheral regions of the limb-rudiments give rise to muscles and contain many more nuclei than the central or cartilaginous region, and hence stain much more intensely than the rest of the section. Comparing the darker peripheral part of the limb-rudiment on the right (injured) side of the embryo with the limb on the normal (left) side, the different regions are found to exactly correspond, showing that there are neither quantitative nor qualitative distinctions between the two limbs. Even in *Amblystoma*, in which an actual reduction in the size of the limb on the right side of the embryo often occurs, owing to the necessary extent of the injury, the peripheral parts of the limb-rudiment are blocked out into the areas in which the various muscles of the upper limb are going to develop; and these regions correspond precisely to those in the limb on the normal side of the body. These facts show that even in those cases where there is a quantitative difference in the two limbs the difference is only one of size and not one of *kinds of tissue* present in the limbs.

Should the constant regeneration of the abdominal muscle-rudiment be urged as an objection to the validity of the conclusions drawn from the experiments, it must be remembered that, although the muscle does regenerate, it nevertheless regenerates



*in front of the limb* and often does not come into contact with it. Even when the regenerating abdominal muscle does come in contact with the limb-rudiment it touches the limb only at its extreme anterior limit, and does not extend throughout the limb-region, as it does in normal embryos. Moreover, the limb on the side of the injured myotomes always keeps pace in development with the limb on the normal side, even from the beginning, its growth being wholly independent of whether the regenerating muscle reaches the level of the limb or not. The constant presence of normal limbs in the injured embryos and the fact that in normal embryos there is no local budding or proliferation of cells from the myotomes or from the abdominal muscle in the posterior limb-region show pretty conclusively that the limbs in the amphibia are of somatopleuric origin.

#### *General Conclusions.*

The ventral processes from the myotomes in the Urodela (Amblystoma and Triton), and in the Anura (Rana and Bufo), go to form the ventral muscles of the body-wall. They do not go into the limbs as "muscle-buds," but pass to the median side of the limb-rudiments. In the Urodela the pronephros develops in the region of the anterior limb. As the pronephric tubules elongate at right angles to the direction of growth of the ventral myotome-processes, the pronephros pushes the myotome-processes over against the limb. The proximity of the two structures is a coincidence, and there is no fusion between the myotome-processes and the anterior limbs in the Urodela. There are no "muscle-buds" in the amphibia such as are found in the elasmobranchs and teleosts. In the posterior limb-region there is no local proliferation from the myotomes to the limbs.

The anterior and posterior limbs in the Urodela and Anura arise as thickenings in the somatopleure. The thickening is formed *in situ* by the multiplication of mesenchyme-like cells, some of which owe their origin to the division of the endothelial cells. The myotome-processes as such take no part in the formation of the limbs. These results obtained from the study



of normal embryos were afterward confirmed by experiments on *Amblystoma* and *Rana*. The myotome-processes were excluded from the posterior limb-region on one side of the body by destroying the lower halves of the myotomes. Even under these conditions the posterior limbs were found to develop normally, although the myotomes and the myotome-derivatives were permanently reduced in size. After injury to the myotomes, the abdominal muscle regenerates, but the injury serves to delay its development and to keep it temporarily away from the limb.

The conclusions reached from the study of the limbs in the normal amphibia, and particularly from the experimental evidence, is that the limbs are of somatopleuric origin; *i.e.*, that the muscles as well as the cartilage and connective tissue of the limbs are formed from the somatopleure, and that the myotome-derivatives are not essential to the formation of muscles in the limbs. This conclusion brings into question the distinction that has been established between muscles derived from the mesothelium and those derived from the mesenchyme. I believe the results of the experiments on *Amblystoma*, and more especially those on the frog, must be interpreted as showing that in these forms, at least, the power to develop striated muscle has not been restricted to the myotomes; *i.e.*, to mesothelium, but that the mesenchyme-like cells of the somatopleure can and do give rise to voluntary muscles in the limbs. The experiments on the myotomes, therefore, furnish additional evidence to that already urged against the conception of a fundamental distinction between mesothelium and mesenchyme.

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## REFERENCE LETTERS.

## PLATES X, XI, AND XII.

<i>Al. C.</i>	Alimentary canal.
<i>B. V.</i>	Blood vessel.
<i>c. l.</i>	Cutis layer.
<i>cart.</i>	Cartilage.
<i>coel.</i>	Coelom.
<i>inj. My.</i>	Injured myotome.
<i>L. L. Rud.</i>	Left limb-rudiment.
<i>L. Rud.</i>	Limb-rudiment.
<i>L. Mus.</i>	Limb-muscles.
<i>My.</i>	Myotome.
<i>Myoc.</i>	Myocoel.
<i>My. pro.</i>	Myotome-process.
<i>P. A. M.</i>	Primary abdominal muscle.
<i>2d P. F.</i>	Second pronephric funnel.
<i>Pron.</i>	Pronephros.
<i>Reg. P. A. M.</i>	Regenerating "primary abdominal muscle."
<i>S. D.</i>	Segmental duct.
<i>S. F.</i>	Somatopleuric fold.

## EXPLANATION OF PLATE X.

(All drawings made with the camera excepting Fig. 17.)

FIG. 1. Cross-section of young *Amblystoma*. Section taken through anterior limb-region, showing beginning of ventral myotome-process, somatopleuric fold of anterior limb, and 2d pronephric funnel.

FIG. 2. Cross-section older larva just behind anterior limb-rudiment.

FIG. 3. Cross-section older larva through anterior limb.

FIG. 4. Cross-section through anterior limb posterior to 2d pronephric funnel.

FIG. 5. Like Fig. 4. Shows myotome-process distinct from limb-rudiment.

FIGS. 6 and 7. Cross-sections through anterior limb-regions of older larvae. Myotome-process seen to median side of limb-rudiment. Connection lost between myotome and ventral myotome-process.

FIG. 8. Cross-section in front of posterior limb-region in *Amblystoma*.

FIG. 9. Cross-section behind posterior limb-region.

FIG. 10. Whole cross-section of *Amblystoma* through anterior limb-region, showing regions indicated in Figs. 1-8.

FIG. 11. Cross-section through anterior limb-region of *Triton*.

FIG. 12. Longitudinal section through posterior limb-region of *Rana*. Section shows abdominal muscle-rudiment passing the limb-rudiment.

FIG. 13. Cross-section through the posterior limb-region of *Amblystoma*.

FIG. 14. Cross-section through the posterior limb-region of *Triton*.

FIG. 15. Cross-section anterior to posterior limb-region of *Triton*. Shows rudimentary abdominal muscle at ventral edge of myotome.

FIG. 16. Whole cross-section of *Amblystoma* through posterior limb-region, showing regions indicated in Fig. 13.

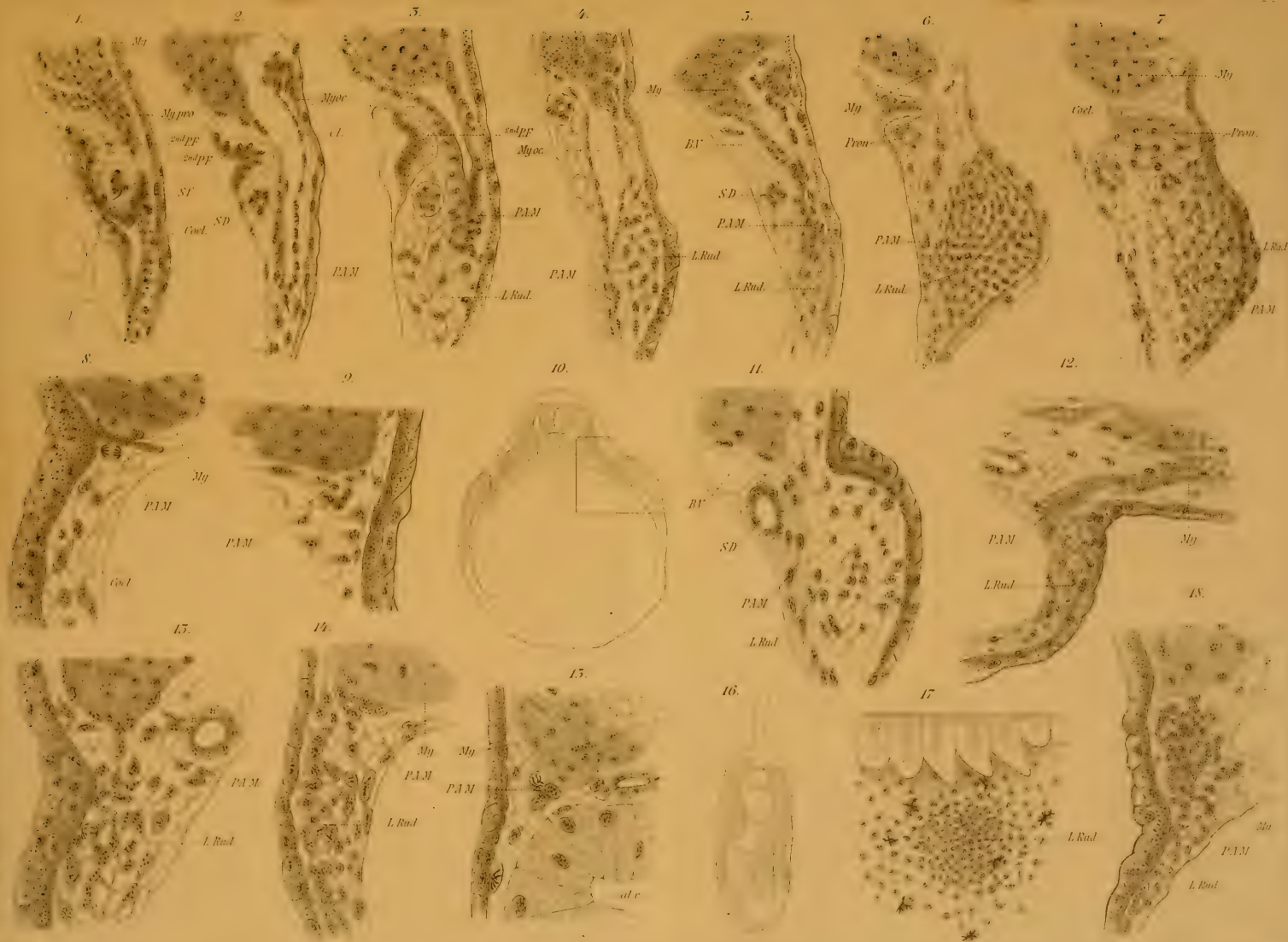
FIG. 17. Whole preparation of anterior limb-region of *Amblystoma*.

FIG. 18. Cross-section through posterior limb-region of *Rana*. (Cf. Fig. 18 with Fig. 12.)















## EXPLANATION OF PLATE XI.

(All outlines traced with the camera.)

FIGS. 19 and 20. Two cross-sections through the posterior limb-region of a tadpole killed four days after injury. Fig. 19 is more posterior than Fig. 20. Both show the ventral edge of the right myotome destroyed; also the posterior abdominal muscle-rudiment. The limb-rudiments are present and uninjured.

FIGS. 21-25 show a series of cross-sections taken through the posterior part of the body of a tadpole killed twenty-six days after injury. Fig. 21 is a section through the posterior limb. The right myotome is reduced and does not come in contact with the limb. Figs. 23-25 show the regenerated posterior abdominal muscle-rudiment.

FIGS. 26-28. Series of cross-sections through the posterior part of the body of an injured tadpole, showing the normal limbs, the reduced myotomes, and the regenerating posterior abdominal muscle-rudiment.

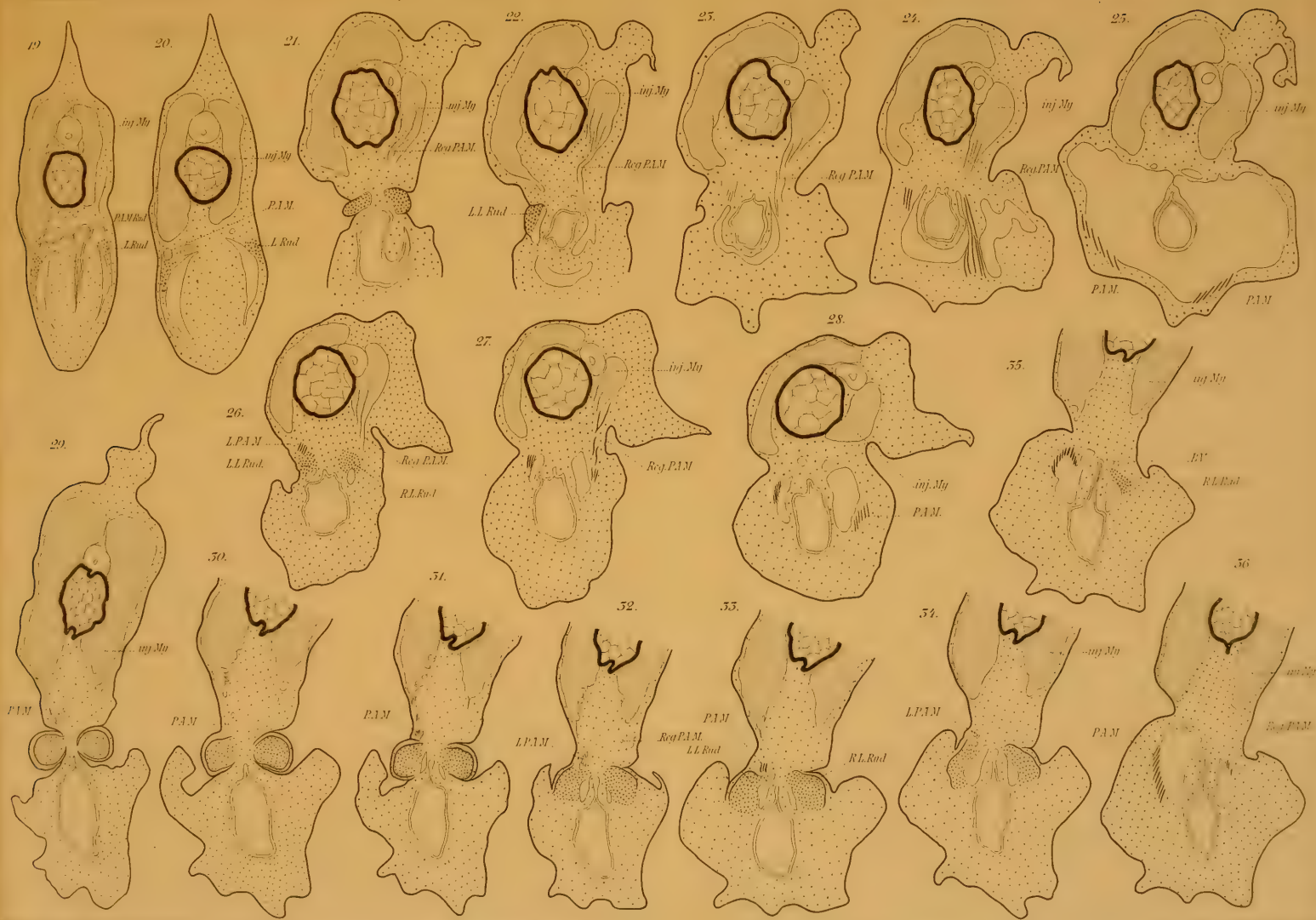
FIGS. 29-35 show cross-sections through the posterior limb-region of a tadpole killed thirty-four days after injury. The limb-rudiments are normal and of equal size. The myotome-derivatives are entirely wanting throughout the limb-region.

FIG. 36 shows a section through the same embryo, but taken in front of the posterior limb-region. The posterior abdominal muscle is just beginning to form, but is greatly reduced.













## EXPLANATION OF PLATE XII.

(All drawings made with the camera.)

FIG. 37. Cross-section through the posterior limb-region of an *Amblystoma* larva killed four weeks after injury. Myotome greatly reduced. Right limb present.

FIGS. 38 and 39. Sections through the right and left posterior limbs, respectively, of an *Amblystoma* larva killed six weeks after injury.

FIG. 40. Cross-section through posterior limb-region of *Amblystoma*. Muscle-regions beginning to develop in the right limb.

FIGS. 41 *a* and 41 *b*. Two cross-sections through the posterior limb-region of a tadpole killed nine days after injury. Fig. 41 *a* more posterior than 41 *b*. Right myotome almost wholly destroyed. Medullary tube wanting. Limb-rudiments equal and normal.

FIGS. 42 and 43. Cross-sections through right and left limb-rudiments of an embryo whose myotome-derivatives were destroyed. Both limbs are beginning to show a condensation of nuclei around the periphery to form muscles.

FIG. 44. Section of Fig. 41 *a*, enlarged, showing relative number of nuclei in right and left limbs.

FIG. 45. Enlarged section of Fig. 33 in Pl. XI. Posterior abdominal muscle wanting on right side of embryo. Right limb normal.

FIG. 46. Cross-section through middle region of right posterior limb of tadpole killed thirty-two days after injury. Posterior abdominal muscle-rudiment wanting. Limb normal.

FIG. 47. Middle region of left posterior limb of same embryo. Comparison of Figs. 46 and 47 shows the two limbs equal, although in Fig. 46 the myotome-derivatives are wanting.











# A CONTRIBUTION TO THE MORPHOLOGY OF DERO VAGA.

HOWARD S. BRODE.

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## I. INTRODUCTION.

THIS paper embodies, in part, the result of three years' work carried on in the Zoölogical Laboratory of the University of Chicago and at the Marine Biological Laboratory at Wood's Holl, Mass.

During the entire time the work has been conducted under the supervision of Dr. C. O. Whitman, Head Professor of Zoölogy at Chicago and Director of the Marine Biological Laboratory, at whose suggestion the work was undertaken. It has been a great source of pleasure to me to be under the direction of so able a teacher, and I am very deeply indebted to him for the interest he has taken in my work.

I desire also to express my gratitude to the authorities of the University of Chicago for the favors which they have from time to time granted me.

My early studies on this annelid were with reference to the process of multiplication by fission. In the course of this study it became evident that a very exact knowledge of the anatomy of one segment was necessary in order to understand fully the changes which take place when a fission zone forms in a segment. This study led to the working out of the nervous system entire and the distribution of the sense organs, together with some points on the general morphology of the worm.

I have made many observations on worms undergoing fission and hope at some future time to bring out a paper on normal and artificial fission and regeneration.

## II. GENERAL REMARKS.

## I. CLASSIFICATION.

*Dero vaga* was originally described by Joseph Leidy in 1880<sup>1</sup> under the name *Aulophorus vagus*. The generic name *Aulophorus* was given by Schmarda<sup>2</sup> in 1861 to a form differing

<sup>1</sup> Jos. Leidy, Notice of Some Aquatic Worms of the Family Naidæ, *Amer. Nat.*, Vol. XIV, No. 6, 1880.

<sup>2</sup> C. Schmarda, Neue wirbellose Thiere, beobachtet und gesammelt auf einer Reise um die Erde (1853-57), Theil I, Heft I, Leipzig, 1861.



somewhat from the form described by Leidy, while the latter form is very closely related to the other species of *Dero* and without doubt should be classed with them.

Vaillant<sup>1</sup> and Beddard<sup>2</sup> classify the annelid as *Dero vaga* Leidy.

The following synopsis is taken from Beddard's monograph:

#### FAMILY NAIDOMORPHA.

*Definition.* — Aquatic Oligochaeta of small size. Setae usually in four groups upon each segment, sigmoid, bifurcate, hastiform, and capilliform. Sexual reproduction at fixed intervals, between which asexual reproduction by fission occurs. Sexual organs (only known in a few types) are situated far forward, commencing even in the fifth segment.

This family of Oligochaeta comprises the following recognizable genera:

- |                                |                                   |
|--------------------------------|-----------------------------------|
| (1) <i>Chaetogaster</i> Baer.  | (5) <i>Pristina</i> Ehrenberg.    |
| (2) <i>Amphichaeta</i> Tauber. | (6) <i>Uncinaiis</i> Czerniavsky. |
| (3) <i>Nais</i> O. F. Müller.  | (7) <i>Chaetobranchus</i> Bourne. |
| (4) <i>Bohemilla</i> Vejdvský. | (8) <i>Dero</i> Oken.             |

GENUS *DERO* Oken. Syn. *Proto* Oersted. *Uronais* Gervais. *Xantho* Dutrochet. *Aulophorus* Schmarda. *Nais* O. F. Müller (in part).

*Definition.* — Dorsal setae capilliform and hastiform,<sup>3</sup> commencing upon the sixth segment. Branchial processes present at hinder end of body. Eyes absent. Inhabit tubes.

He notes eight species as follows:

- |                                |                                    |
|--------------------------------|------------------------------------|
| <i>D. mülleri</i> Bousfield.   | <i>D. limosa</i> Leidy.            |
| <i>D. furcata</i> Oken.        | <i>D. perrieri</i> Bousfield.      |
| <i>D. obtusa</i> D'Udekem.     | <i>D. vaga</i> (Leidy).            |
| <i>D. latissima</i> Bousfield. | <i>D. multibranchiata</i> Stieren. |

*DERO VAGA* (Leidy). *Aulophorus vagus* J. Leidy. *Am. Nat.*, 1880, p. 423. *D. vaga* L. Vaillant. *Annelés*, p. 383.

*Definition.* — Length about 8 mm.; number of segments, 25. Body ending in two long processes; branchiae rudimentary, only two slight processes. Dorsal setae bundles consisting of one capilliform and two pectinate setae.<sup>4</sup> Perivisceral corpuscles present. Contractile hearts in VIII, IX, and X.

*Hab.* — North America; Trinidad.

<sup>1</sup> L. Vaillant, *Histoire naturelle des Annelés Marins et d'Eau douce*, Tome III, Paris, 1889.

<sup>2</sup> F. E. Beddard, *Monograph of the Order Oligochaeta*, Oxford, 1895.

<sup>3</sup> Palmate setae may also be present in the dorsal bundles.

<sup>4</sup> The setae in the dorsal bundles are more nearly palmate than pectinate, and there are usually two capilliform and two palmate in a bundle. Occasionally three of each are present. (Notes are mine.)

## 2. NATURAL HISTORY.

The family Naidomorpha includes many minute transparent worms varying in length from 1 to 15 mm. Some members of the family may be found in almost every collection of water plants from a pond or ditch.

Members of the genus *Dero* may be recognized at once by the presence of digitiform processes at the posterior end of the body (Pl. XIII, Fig. 2), and the most apparent distinguishing character of *Dero vaga* is its habit of building a case for itself and pulling it about on the surface of the water (Pl. XIII, Fig. 1).

Specimens may be found in ditches, ponds, and small lakes where there is an abundance of vegetation. For this work collections were made at Glacialis Pond near Cambridge, Mass., and at Wolf Lake, Ill. The surface of the Cambridge pond was almost covered with *Lemna*, and the worms were remarkably abundant. The worms prefer a pond in the open field, but are found most abundantly in the shade of leaves of water plants near shore. In case there is a lack of *Lemna* the worms may be found in algae below the surface or even on the bottom of the pond.

*Dero vaga* varies in length from 5 to 10 mm., according to the progress of the growth preceding fission. The width never exceeds .25 mm. The number of segments may vary from twenty-five to sixty. All segments excepting the first five have four bundles of setae. The first segment (prostomium) has no setae. Segments II-V have ventral setae only.

The anterior end of the body is slightly enlarged, and during locomotion the pharynx is everted to form a sucking disc. The branchial apparatus at the posterior end of the body is made up of a disc with undulating edges, which occupies a dorsal position, and two well-defined digitiform appendages which are found near the ventral side. In addition to these parts there are two long, slender, outwardly curving appendages with enlarged tips which arise below the other parts (Fig. 2). All parts of this apparatus are covered densely with cilia, which, during life, are in constant motion. When the worm is undis-

turbed this whole apparatus is protruded from the case and is turned with the concave side of the disc uppermost. When the apparatus is contracted the disc is so folded as to give the appearance of four lobes, which, with the two well-defined ventral lobes, make up the half-dozen blunt papillae described by Leidy.

The case (Fig. 1) consists of a thin hyaline tube covered over with dead *Lemna* leaves, statoblasts of fresh-water Bryozoa, *Arcella* shells, or small pieces of almost any substance which may be floating on the surface of the water in which they live. As the worm grows longer the case is also increased in length, and when fission is complete the worms place their heads together at the middle of the case and break it in two. Each worm goes away with one half of the old case. The cases of worms found on the surface of the water will float when the worm is driven out, while those found on the bottom will sink under similar conditions. Worms have been observed to change their position gradually from surface to bottom and from bottom to surface, according to the location of the food supply.

Locomotion is effected in a jerking manner by extending the body some distance out of the case and attaching the anterior portion by means of the pharynx and the ventral setae and then contracting the body, pulling the case forward. At the time of the development of the sexual organs the worms have been observed to leave their cases and crawl about on the bottom of the dish.

The food taken is apparently entirely vegetable matter and consists of desmids, algae, and at times the fronds of *Lemna* and *Wolffia*.

Sexual organs are developed during the first two weeks in July. However, worms kept over winter in an aquarium showed sexual organs as early as April 1. A clitellum is formed which covers segments V-VII. Spermathecae occur in segment V and the atrium of the sperm duct is plainly visible in segment VI. Egg masses fill a large part of the body cavity posterior to the clitellum. Eggs outside of the body have not been found and the manner of laying the eggs is unknown to me.

At all seasons of the year excepting two weeks in July the worms multiply by fission. This takes place slowly in the winter and very rapidly during the summer. The new head and tail form almost completely in the region of fission before separation takes place. In the summer fission occurs as often as three times a week. In a dish in which one worm was placed, eight were found at the end of one week. At the end of two weeks fifty were counted.

The fission zone is formed near the middle of a segment and not between two segments, as has been described for some other Naidomorpha. In so far as I have observed, the regeneration of head and tail in cases of fission occurs in the one somite in which the fission zone first appears. The number of segments formed in the new head is constant, being five, while the number in the new tail varies, — in fact there is no limit to the growth of the tail. Usually twelve to sixteen segments are visible before second fission occurs. When fission occurs in an anterior individual the zone appears in the first new segment of the previous fission zone.

Three fission zones may be present in one worm during the period of rapid multiplication. More than three have not been observed by me.

At the first outward signs of fission the worm has from thirty to forty segments and measures from 5 to 6 mm. in length. When the worms are ready to separate there are from fifty to sixty segments, and the length has increased to 10–11 mm. The number of the segment in which fission occurs is fairly constant, but is liable to vary. In all observed cases it occurs back of segment XVII and usually anterior to segment XXII.

If a worm is divided by cutting, both parts will continue to live and in a short time will regenerate a new head and tail and form perfect individuals. There seems to be some limit, however, to the number of segments which will regenerate a new head or tail.

My experiments have not been extensive enough to establish any rule regarding regeneration, but they have shown that the number of segments regenerated at the anterior end of the



body is constant, *i.e.*, only enough are regenerated to complete the five first segments. If two segments are removed a like number will be regenerated, but if seven segments be removed there will be but five new segments formed. In the latter case the regeneration proceeds much more slowly than in cases where fewer segments are removed. In order that the removed anterior portion of a worm regenerate a tail it seems to be necessary for it to have at least three or four segments in addition to the five in the cephalized part.

In case a cut is made a short distance in front of a fission zone the part anterior to the zone may disintegrate while the posterior individual continues to live, and the normal process of regeneration is apparently hastened to adjust the worm to the new conditions.

### 3. METHODS.

The following methods were found to give good results:

*Killing and Hardening.* — Hot corrosive sublimate (saturated aqueous solution); hot acetic corrosive sublimate, Hermann's fluid, and 1/10% osmic acid.

*Maceration.* — 1/10% nitric acid, also a mixture of glycerine, acetic acid, and water, equal parts. The worms treated with the nitric acid were dissected with needles ground flat.

*Staining.* — Of the ordinary stains Delafield's haematoxylin, Böhmer's haematoxylin, Grenacher's borax carmine, and the triple stain of Heidenhain-Biondi-Erlich gave good results.

For the working out of the nervous system and sense organs the following methods were used:

*Gold Chloride.* — The method employed was essentially the same as that given by Mr. C. L. Bristol in the *American Naturalist* for September, 1894. The live worms were killed in 10% formic acid and left for one minute, after which they were placed in 1% aqueous solution of gold chloride for ten minutes, and then left in 1% formic acid for from two to four hours. The gold chloride solution was kept out of bright sunlight while the specimens remained in it. The reduction in 1% formic acid was carried on for a portion of the time in sunlight.



*Methylen Blue.*—A solution of the stain was made by dissolving a small amount of the powder in water; a very small drop of this was added to a drop of clear water on the slide in which was a live worm. A cover glass supported on wax feet was placed over this and the examination was made with the compound microscope. Within two hours the peripheral nervous system would be well stained. Specimens were also killed by adding a drop of 2% formalin to the slide on which they were placed. The methylen-blue solution was then added. This gave the best results in the study of the sense organs, while the peripheral nerves took the stain only when the specimen was alive.

*Imbedding.*—Specimens were imbedded in paraffin and were cut from  $5\ \mu$  to  $15\ \mu$  thick.

*Drawing.*—Drawings were made by aid of the Zeiss-Abbé camera lucida, and a number of points were worked out by means of reconstructions and composite drawings from camera sketches. The majority of the figures were drawn at a magnification of 400 diameters, and all were reduced one-half in reproduction.

### III. MORPHOLOGY.

#### 1. THE BODY WALL AND EXTERNAL CHARACTERS.

##### (a) *Segmentation.*

The number of segments in an individual depends upon its condition. If sexually mature it may have from twenty-five to thirty-five. This period is so short that the growth in length in the tail region is scarcely interrupted. If the worm is multiplying by fission it may have as many as sixty segments.

The first segment differs from the others in appearance. This difference may be due to its position and modified function. The well-developed branchial apparatus at the posterior end of the body in all probability does not represent a segment, for in cases of fission it is not fully formed until many segments have been marked off anterior to it. The growing zone, in which are located the cells corresponding to the teloblasts of the embryo, lies just anterior to this branchial area. The

segments in the anterior region are shorter than those following, and all the segments have a more or less distinct secondary annulation.

(b) *Setae*.

The first segment bears no setae. The second, third, fourth, and fifth bear each two bundles ventrally, containing from eight to twelve long slender setae with bifurcate tips. The following segments bear four bundles — two dorsally and two ventrally. The dorsal bundles contain two peculiar palmate setae and two capilliform setae. The ventral bundles contain four or five bifurcate setae resembling those in the anterior segments, but being somewhat shorter and thicker. In sexually mature individuals the ventral setae are absent in the sixth segment.

In cases of fission and regeneration the ventral setae form before the dorsal, and the setae in the anterior segments whether in the head or tail are always the first formed.

(c) *Epidermis*.

The epidermis is composed principally of hexagonal columnar cells, the width of which is about twice the height. These cells have large nuclei and are covered externally by a thin chitinous cuticle.

In addition to these cells there are found sensory cells and gland cells. The sensory cells are spindle-shaped and have large nuclei compared to the size of the cell. The nuclei stain much more deeply than those of the ordinary epidermal cells. From the outer end of the cell projects a long, stiff hair or bristle (Figs. 10, 14-16). The sensory cells may occur singly or in groups. In the latter case the epidermis shows a hemispherical elevation at that spot. The sense organs will be discussed in more detail later on in this paper.

The gland cells are goblet-shaped and often are found with granular contents. They are more abundant in the head region than elsewhere.

The epidermis is thicker on the ventral side than on the dorsal and the cells multiply rapidly where a fission zone forms and at the posterior end of the worm. The epidermis of the

prostonium is very much thickened and is thickly set with sensory cells.

(d) *Epidermal Glands.*

In addition to the scattered gland cells mentioned above there are some other modifications of the epidermis which very probably are glandular in their nature.

The clitellum covering segments V-VII during the time of sexual development has not been specially studied in this form, but the structure and function is in all probability much the same as has been described in other Oligochaetes.

Near the posterior border of every segment beginning with segment VI there is a marked band of cells in the hypodermis (Pl. XIV, Fig. 10, *gl.b.*) which when treated with ordinary reagents appear as empty cells surrounded by a substance taking a stain somewhat more deeply than other parts of the epidermis.

Another peculiar band (Pl. XIV, Fig. 10, *d.gr.*) appears in specimens stained with methylen blue. It is situated just posterior to the anterior girdle of sense organs and consists of very deep-staining dots regularly arranged on all segments back of the first five. Connecting the dots in the band there appears a narrow groove in the epidermis.

I have not made a careful study of these structures, and my reason for mentioning them is on account of their metameric arrangement.

(e) *Muscles.*

There are two layers of muscles in the body wall, an outer circular and an inner longitudinal layer. The muscles are all of the so-called Nematoid type.

The circular muscles (Pl. XIV, Fig. 18, *c.mus.*) are arranged in a single layer just beneath the epidermis. The nucleated plasma parts are gathered in the two lateral lines (Pl. XIV, Fig. 18, *ll.*) of the worm lying in the breaks between the dorsal and ventral halves of the longitudinal muscles. This peculiar arrangement of nuclei was first made out by Hesse,<sup>1</sup> and I

<sup>1</sup> R. Hesse, Beiträge zur Kenntnis des Baues der Enchytraeiden, *Zeit. f. wiss. Zool.*, Bd. LVII, 1893, p. 6.

have been able to corroborate his statements so far as concerns *Dero* and *Tubifex*.

The longitudinal muscle layer (Pl. XIV, Fig. 18, *l.mus.*) lies next the circular and is composed of fibres which are not arranged in groups, as in *Lumbricus*, but are isolated. The plasma parts with their nuclei show on the inner side of the layer.

## 2. NERVOUS SYSTEM.

### (a) *Historical.*

Observations have been made on *Dero* by a number of investigators, but little has been written concerning the nervous system. Perrier<sup>1</sup> described the nervous system of *Dero obtusa* (*D. perrieri*), but confined his observations to the dorsal ganglion and the ventral cord. No account was given of lateral nerves.

Reighard<sup>2</sup> described the nervous system of *D. vaga* and gave an account of the nerves arising from the dorsal ganglion and commissure. No mention was made of lateral nerves from the ventral cord. He noticed the "lateral line," but could not trace it as far forward as the dorsal ganglion.

In 1885 Anton Štolc<sup>3</sup> in a paper on the anatomy and histology of *D. digitata* Müll. describes and figures the nervous system in greater detail. The dorsal ganglion in the form studied has a greater length from front to back than has that of *D. vaga*. The ganglia of the ventral cord correspond in shape to those of *D. vaga*. He figures some very minute nerves from the anterior face of the dorsal ganglion between the two large nerves and also some from the inner sides of the commissures near their posterior place of fusion. He cites similar structures in *Stylaria* described by Vejdovský. I am inclined to doubt the nervous nature of these fibres, for muscles are found in similar positions and I have been unable to find nerves

<sup>1</sup> Edmond Perrier, *Histoire Naturelle du Dero obtusa*, *Archives de Zoologie expérimentale et générale*, Tome I, 1872, pp. 83-85.

<sup>2</sup> J. Reighard, On the Anatomy and Histology of *Aulophorus vagus*, *Proc. Am. Acad. Arts and Sci.*, Vol. XX, 1884, pp. 101-104.

<sup>3</sup> A. Štolc, *Dero digitata* O. F. Müller: *Anatomická a Histologická Studie. SB. Böh. Ges.*, 1885, pp. 65-95. 2 pl.



so situated in *D. vaga*. He does not figure the nerves from the commissure corresponding to those found by Reighard. He found that as many as three nerves were given off from the ventral cord in a segment. These correspond in position to some of the nerves found in *D. vaga*.

(b) *Descriptive.*

The nervous system of *Dero vaga* consists of a central ganglionated cord with lateral nerves and a sympathetic system which covers the pharynx.

*Central Nervous System.*—The central cord is made up of a dorsal ganglion, the so-called "brain," and an indefinite number of ventral ganglia, corresponding in number to the segments of the body. The dorsal ganglion is united with the first ventral ganglion by a commissure, which passes around the alimentary canal. The dorsal ganglion (Pl. XIII, Figs. 3, 6, *d.g.*) is situated in the first segment (preoral lobe) and consists of two pear-shaped masses united at their larger ends, while the smaller ends are drawn out to form the commissures. The ventral ganglia in the first four setigerous segments are crowded together so that no space is left between them. However, the characteristic lobed appearance clearly shown in the following ganglia may also be traced in this part of the cord. The ventral cord consists of two large bundles of fibres on which are found at intervals of one segment the masses of ganglion cells which form the ganglia. An intermediary nerve is also clearly visible.

The typical ganglion (Pl. XIII, Figs. 6, 8) consists of a series of four distinct enlargements. The second is the largest and is near the middle of the ganglion. The entire mass, with the exception of the posterior enlargement, lies in one segment and occupies a position a little posterior to the middle. The posterior enlargement occupies a position close to the dissepiment, and apparently has been pushed back so as to lie in the next segment.

At the tail end of the worm or at a fission zone, the ventral cord is in connection with the epidermis (Pl. XIII, Fig. 7), from



which it is constantly being formed. I have observed no specimens in which this connection did not exist. Sexually developed forms as well as those undergoing fission were observed. In consequence of this continued growth the ganglia vary in number.

A cross section of the ventral cord through the widest part is shown highly magnified in Pl. XIV, Fig. 17. The nerves emerging from the cord (*l.n.*) are those which pass through the setae bundles. The ganglion cells (*g.c.*) are arranged in three groups, two lateral and one ventral. The fibrous portion (*fb.*) is divided by faint clear spaces into three parts. In the lower portion of the middle part, the cross section of the intermediary nerve (*i.n.*) may be seen, and in the dorsal part of the fibrous portion are three giant fibres (*g.f.*). Two muscle bands (*mus.*) appear, one on either side of the fibrous bundle, and a blood vessel (*b.v.*) lies closely applied to the dorsal surface of the cord. The examination of dissected specimens shows large irregular cells scattered along the dorsal surface of the cord. In the tail region and at a fission zone these cells are very much more abundant. Without doubt they correspond to the "chorda cells" of Semper,<sup>1</sup> and are probably identical with the "neoblasts" described by Miss Randolph<sup>2</sup> in *Lumbriculus*.

In describing the lateral nerves I shall begin with the simpler condition found in the body segments, and proceed later to describe the nerves in the so-called "head."

As has been mentioned before, each segment of the body contains a ganglionic swelling of the ventral cord. This ganglion reaches its maximum width at a point posterior to the middle of the segment, and a portion of the swelling extends through the dissepiment into the next following segment. Four pairs of lateral nerves are given off from each ganglion (Pl. XIII, Figs. 3, 6). These nerves pursue essentially the same course. On leaving the cord they pass obliquely downward and away from the cord, and pass through the longitudinal muscle layer

<sup>1</sup> C. Semper, Die Verwandtschaftsbeziehungen der gegliederten Thiere, III, Strobilation und Segmentation, *Arbeit. a. d. Zool.-Zoot. Inst. Würzburg*, Bd. III, 1876, p. 186.

<sup>2</sup> Harriet Randolph, The Regeneration of the Tail in *Lumbriculus*, *Journ. of Morph.*, Vol. VII, 1892.

and come to lie among the circular muscle fibres. At the point where the nerve enters the muscle layers a branch is given off which passes to the ventral side of the body, while the main trunk passes dorsally. The nerves pursue a straight course around the body, and no branches have been noticed from any of the nerves in the body region.

The first nerve from the ganglion (*l.n.<sup>1</sup>*) passes around the body near the middle of the segment. It is the second largest of the four, and apparently innervates the muscles and other organs of the viscera. The second nerve (*l.n.<sup>2</sup>*) passes through the setae bundles, and is the largest nerve of the group. It innervates the greater band of sense organs found on the posterior part of the segment. The third and fourth nerves (*l.n.<sup>3</sup>*, *l.n.<sup>4</sup>*) are smaller than the first and are nearly equal in size. The third passes into the dissepiment, and very probably innervates the muscles of its walls. The fourth lies in the following segment, and supplies the sense organs in the lesser band which encircles the body close to the anterior end of the segment. This arrangement of nerves I have traced forward through all the segments up to the first.

The nerves from the dorsal ganglion are four in number, and, like those from the following ganglia, are placed three in the first segment and one in the next following or second segment. This latter, fourth nerve, has a course corresponding to that of the following nerves, while the three anterior nerves have a varied course. The first nerve (*n.<sup>1</sup>*) is large and leaves the ganglion dorsally and laterally at a point near where the commissure begins its downward course. It grows out a short distance as a single nerve, and later breaks up into three branches. The first runs almost straight forward, while the second bends below the first and follows the anterior wall of the proboscis, approaching the corresponding branch from the other nerve of the pair at the median line. The third branch passes forward and downward, and extends to the epidermis. Each of these branches subdivides into smaller branches near the body wall. The second and third nerves (*n.<sup>2</sup>*, *n.<sup>3</sup>*) arise close together a short distance below the first. The second soon divides into two rami and these pass to the ventral and

lateral walls of the proboscis. The third nerve passes downward and slightly forward, and lies close to the anterior wall of the buccal cavity. From the distribution of the first three nerves it seems probable that some fibres at least in each of them are sensory. The fourth nerve (*n.4*) is situated at some distance from the third, but I have been able to trace ganglion cells from the dorsal ganglion as far down the commissure as the place of origin of this nerve.

(c) *The Sympathetic System.*

Two systems of visceral nerves have been described under the name "sympathetic nerve." Leydig<sup>1</sup> distinguished the two by the names "sympathetic" and "vagus." The system covering the pharynx he called the vagus nerve, and applied the term "sympathetic" to the system distributed over the intestine. Other writers speak of his vagus as the "sympathetic of the head" or simply as "sympathetic nerve."

The sympathetic in this latter sense has been described in many annelids, both marine and fresh-water. Beddard says:<sup>2</sup> "I have never found it to be wanting in any earthworm where I have looked for it."

Vejdovský has described this system in Chaetogastridae and also mentions having found a ganglion on the posterior part of the pharynx of a young specimen of *Nais elinguis*. So far as I know, this is the extent of the work on this system in the Naidomorpha.

The pharynx of *Dero vaga* occupies the first four setigerous segments, and is composed of two very unlike regions. The dividing line is the boundary between the second and third setigerous segments. The lumen of the anterior half is divided into a dorsal and a ventral part by an infolding of the wall on either side (Pl. XIII, Fig. 5).

The main branches (Pl. XIII, Fig. 5, *m.s.n.*) of the sympathetic (vagus of Leydig) nerve lie in the groove between these two divisions and extend back to the ganglia (Pl. XIII, Fig. 4,

<sup>1</sup> F. Leydig, Ueber den Bau des thier. Körpers, Tübingen, 1864.

<sup>2</sup> Beddard, *l.c.*, p. 20.

*s.g.*), which are situated on the sides of the pharynx at the junction of the anterior with the posterior region. The connecting nerves are distributed over the dorsal part of the pharynx, the whole system lying just beneath the epithelium of the canal.

The main trunks (*s.n.*) are given off from the inner sides of the oesophageal commissure and from points a little below the brain. Their course is slightly dorso-lateral until they reach the pharynx, where they divide into two branches; the larger (*m.s.n.*) takes the position in the groove above alluded to, and the smaller extends upwards and back over the side of the pharynx and unites with the larger branch at the ganglion.

At the place of branching, a large commissural nerve joins the two main trunks. A large nerve also extends over the dorsal side of the pharynx, uniting the two ganglia. Between these two commissures there are about fifteen smaller nerves, which also extend over the pharynx, anastomosing with the upper longitudinal trunks.

The ganglia (*s.g.*), two in number, are double, the ganglionic mass being divided into an anterior and a posterior half. These parts are elongated dorso-ventrally, and the anterior mass is smaller than the posterior.

Sensory cells are found in the pharyngeal epithelium, which in some cases are apparently connected with the branches of the sympathetic nerve. These cells are especially numerous along the two main nerve trunks.

No connection has been found to exist between the sympathetic system and the nerves from the ventral cord, and no indication of a sympathetic nerve in Leydig's sense has been observed.

(d) *Comparative.*

The nervous system in all *Oligochaetes* consists of a dorsal ganglion lying above the alimentary canal, and a ventral ganglionated cord connected with it by a circum-oesophageal commissure.

The dorsal ganglion may be found in the first segment or it may be pushed back to lie in the third segment. In the development of the earthworm the brain is formed in the first



segment, and, as development proceeds, it is pushed back to the third. From this we may infer that the former condition is more primitive than the latter. The shape of the dorsal ganglion differs in different species. In *Dero vaga* it is very simple, much more so than in other Naidomorpha and in Tubificidae, and resembles somewhat the dorsal ganglion in Lumbricidae.

From descriptions of the nerves given off from the dorsal ganglion the number varies greatly. This may be due largely to insufficient investigation and difference in interpretation as to the origin of the nerves found. I think it highly probable that the number of nerves given off from the dorsal ganglion in any case will be found to correspond with the number given off from each ventral ganglion. This correspondence exists in *Dero* and Beddard<sup>1</sup> states that a similar condition exists in *Spirosperma*. In most earthworms there appear to be three nerves from the dorsal ganglion and three from each ventral ganglion. The third nerve in each segment, however, sends off a branch which goes to the dissepiment. Further investigation is necessary before we can compare the nerves of the dorsal ganglion of the earthworm with the lateral nerves from the ventral cord.

I believe that the arrangement of nerves which I have described for *Dero vaga* will be found to hold good for the majority of forms in the families Naidomorpha and Tubificidae. The observations of Vejdovský<sup>2</sup> and Štolc<sup>3</sup> on Tubificidae point in this direction. While Vejdovský figures five nerves to a segment, it is not improbable that the two going to the dissepiment are really one. Štolc describes one to the dissepiment and three others within the segment.

<sup>1</sup> Beddard, *l.c.*, p. 20.

<sup>2</sup> F. Vejdovský, *System und Morphologie der Oligochaeten*, Prag, 1884, p. 85. Pl. VIII, Fig. 2.

<sup>3</sup> A. Štolc, *Ilyodrilus coccineus* Vejd., Ein Beitrag zur Kenntnis der Tubificiden, *Zool. Anz.*, Bd. VIII, 1885, p. 641.



### 3. SENSE ORGANS.

#### (a) *Descriptive.*

In common with other Oligochaetes the epidermis of *Dero vaga* is richly supplied with sense cells. These cells may occur isolated or in well-defined groups of from five to seven cells each.

The isolated cells are scattered irregularly over the entire body and are more numerous on the anterior segments. On the first five or six segments there are at least three hundred of these cells to a segment. On the tenth segment and on the following segments the number is near fifty.

The groups of cells, which I shall speak of as sense organs, have a very definite arrangement. They are found on every segment of the body and, excepting the first five segments, are arranged in two bands encircling the segment. The lesser band is found near the anterior part of the segment and the greater band passes through the setae in the posterior part of the segment. In the first segment the sense organs are very numerous and are irregularly distributed. The largest organs are on the anterior and dorso-lateral borders of the segment. At the dividing line between the first and second segments there is a band of organs, the exact number in which is not easily determined on account of the great number of isolated sense cells which occur on these segments. However, enough can be seen to determine that the organs are less numerous here than in the double bands in the next following segments, and, furthermore, that such organs as can be made out correspond in position to those in the lesser bands of the trunk segments. Segments II-V at first sight seem to possess but one band of organs, which occupies the position of the greater band in the other segments. Closer study shows that these organs are not exactly in the same line around the segment; and, taking into account the fact that the number of organs in one of these bands corresponds to the number in the two bands of the other segments, I think we are justified in assuming that this band is a double band formed by the union of the greater and lesser band.

There are twelve well-defined organs in the greater band, six large and six small (Pl. XIII, Fig. 9; Pl. XIV, Figs. 11-13). In the lesser band there are eight organs, two large and six small. These organs have a very constant, regular arrangement in each band so that the organs in corresponding bands form longitudinal rows which extend the whole length of the body,—in all twenty rows.

The rows containing the larger organs are the two median-dorsal rows (*m.s.o.*), the two dorso-lateral (*d-l.s.o.*) occurring just below the dorsal setae, the two lateral rows (*l.s.o.*) occupying a position just below the so-called "lateral line," and the two ventro-lateral rows (*v-l.s.o.*) which are found immediately above the ventral setae. The lateral rows only are made up of organs occurring in the lesser bands.

The sense organs in the living worm appear as hemispherical elevations of the epidermis and are set with hairs. The diameter of these elevations varies from .0175 to .025 mm., and the elevation above the surface is about .0125 mm. The hairs have a length of .0175 mm. After the animals have been subjected to the killing reagents, the organs are found to be, in most cases, level with the surface of the epidermis.

The organs are very simple in their structure, consisting of sensory cells alone. Covering cells have not been observed. The sense cells (*s.c.*) are elongate with enlarged middle portion and have large nuclei which stain deeply with the ordinary reagents, but remain clear when treated with gold and silver. Each cell bears a long stiff hair. The inner end of the cell in many cases rests directly on the lateral nerve from the ventral cord (Pl. XIV, Fig. 16, *l.n.*); otherwise no direct connection has been observed between cells and nerves.

The sense organs in the greater band occur on the second lateral nerve (*l.n.*<sup>2</sup>), while those in the lesser band are found in connection with the fourth lateral nerve (*l.n.*<sup>4</sup>). The first well-defined band of organs on the body is innervated by the fourth nerve from the dorsal ganglion.

(b). *Comparative.*

*Oligochaetes.* — In this discussion of the sense organs in the various forms I shall confine my attention to those organs which are distributed over the surface of the body, generally termed “touch organs,” and which are diffusely or metamerically arranged.

Vejdovský<sup>1</sup> summarizes the discoveries made prior to 1884, and classifies the various forms of “touch organs” as touch papillae (“Tast papillen”), touch hillocks (“Tasthügel”), and cup-shaped organs (“becherförmigen Organen”). The “touch papillae” are organs similar to those found in *Chaetogaster*. The “touch hillocks” are non-retractile elevations such as are found on *Nais appendiculata*. The “cup-shaped organs” have been described in Lumbriculidae and Lumbricidae.

These organs which Vejdovský has classed in three groups are much alike. “Touch papillae” and the “cup-shaped organs” seem to be names applied to organs both of which may appear either as papillae or as insinkings of the skin. They are essentially identical in structure. The “touch hillocks” differ from the other organs in that they are not retractile. They usually occur in forms which have a covering of debris over the surface of the body. The sense organs project through this covering.

Miss Randolph<sup>2</sup> has described organs in some Tubificidae which she considers intermediate between the “touch papillae” and the “touch hillocks.” Bousfield<sup>3</sup> considered that the genus *Ophidonais* should be united with that of *Slavina* on the ground of similarity of sense organs. In reply to this, Štolc<sup>4</sup> asserts that the organs of *Ophidonais* differ much from those of *Slavina*, the former being “touch papillae,” while the latter are “touch hillocks.” However, it can hardly be said that they

<sup>1</sup> Vejdovský, *l.c.*, pp. 96-99.

<sup>2</sup> Harriet Randolph, Beitrag zur Kenntnis der Tubificiden, *Jenaische Zeit. f. Naturg.*, Bd. XXVII, N.F. XX, p. 465.

<sup>3</sup> E. C. Bousfield, *Slavina* and *Ophidonais*, *Jour. Linn. Soc.*, XIX; *Jour. Royal Mic. Soc.*, 1886, p. 445.

<sup>4</sup> A. Štolc, Beiträge zur Kenntnis der Naidomorpha, *Zool. Anz.*, Bd. IX, 1889, p. 503.

differ in histological structure. The only difference lies in the fact of the different external appearance of the organs. However, since it is probable that all the Oligochaetes possess "touch organs," the fact of their presence alone cannot be used as a character in determining relationships in genera and species.

(c) *Metameric Sense Organs.*

*Oligochaetes.* — Segmentally arranged sense organs have been described in *Nais appendiculata* and *Nais lurida*. In the former they are said to be arranged in a band passing through the setae. There are from fifteen to twenty organs in a band. In the latter they are said to be in two bands made up of six to eight organs each. The arrangement of organs in *N. appendiculata*, as figured by Vejdovský,<sup>1</sup> is very similar to that in *Dero vaga* in the band passing through the setae.

Miss Randolph<sup>2</sup> has described metameric organs in two species of Tubificidae where they are arranged in two bands, one through the setae and one near the dissepiment. In one species there is a showing of a third band. She does not mention the number of organs in a band nor give their arrangement.

Vejdovský<sup>3</sup> mentions a single pair of organs in every segment in *Lumbriculus*, *Claparedilla*, and *Rhynchelmis* in the lateral-line region. In *Benhamia*, Eisen<sup>4</sup> figures a band of sense organs about the middle of every segment.

Miss Langdon<sup>5</sup> and Hesse<sup>6</sup> both describe three bands of sense organs to a segment in *Lumbricus*. Hesse finds the number of sense organs to be the same on the two lateral halves of the body, there being as many as one hundred in the middle

<sup>1</sup> Vejdovský, *l.c.*, Taf. III, Fig. 17.

<sup>2</sup> Harriet Randolph, *Jenaische Zeit. f. Naturg.*, Bd. XXVII, N.F. XX, pp. 463-476.

<sup>3</sup> Vejdovský, *l.c.*, p. 98.

<sup>4</sup> Gustav Eisen, *Pacific Coast Oligochaeta*, II, *Cal. Acad. Sci.*, Vol. II, No. 5. Pl. XLVII, Fig. 20.

<sup>5</sup> Fanny E. Langdon, *The Sense Organs of Lumbricus agricola*, *Journ. of Morph.*, Vol. XI, pp. 193-234.

<sup>6</sup> R. Hesse, *Zur vergleichenden Anatomie der Oligochaeten*, *Zeit. f. wiss. Zool.*, Bd. LVIII.



or largest girdle. These organs vary in size, but he does not describe any arrangement of organs in rows longitudinally.

*Polychaetes.* — In this group of annelids we have the well-known “lateral organs” of capitellids which have been described at length by Eisig.<sup>1</sup> These number one pair to a segment and are situated in the lateral-line region of the body, and in a band passing through the setae.

Similar organs have been found by Meyer<sup>2</sup> in *Polyophthalmus*, and in addition to these, in this form, there are sense organs resembling eyes on twelve of the body segments. These are in a line with the other organs, but are situated near the anterior border of the segment.

Eisig finds in addition to the “lateral organs,” which are highly developed, some organs of a simpler character which are scattered diffusely over the body. These he calls “cup-shaped organs.” He thinks that for the present we can assert no relationship between these two kinds of organs excepting the fact of their origin from the epidermis.

*Leeches.* — The metameric sense organs, sensillae, of leeches have been described by Whitman.<sup>3</sup> In *Clepsine* there is a band of fourteen organs around the anterior portion of every segment. These organs are so situated as to form longitudinal rows extending the whole length of the leech. In six rows the organs are larger than in the others. In *Clepsine* the organs of the median dorsal row gradually change from tactile to visual organs in the anterior segments.

*Vertebrates.* — In fishes and larval batrachians we find scattered “cup-shaped organs,” and also more highly developed organs which are arranged in certain definite lines, and in young forms often are arranged metamerically. These organs are considered by some writers to be essentially the same, — the variations being due to difference in development rather than to a structural unlikeness. The suggestion has been made by several workers that there is a homology existing between these

<sup>1</sup> See Bibliography, p. 173, Nos. 8 and 9.

<sup>2</sup> Eduard Meyer, *Zur Anatomie und Histologie von Polyophthalmus pictus* Clap., *Archiv f. mikros. Anat.*, Bd. XXI, 1882, pp. 797-799.

<sup>3</sup> C. O. Whitman, *The Metamerism of Clepsine*, *Festschr. f. Leuckart*, Leipzig, 1892, pp. 385-395.



organs and the sense organs of worms, but from the present status of our knowledge of the subject we cannot draw any valuable conclusions.

#### 4. THE SO-CALLED "LATERAL LINE."

##### (a) *Historical.*

This structure has been a problematic one for twenty years, and I think that at last the true nature of the "lateral line" of annelids has been discovered. With this thought in mind I give below an extended historical account setting forth the various views that have been held regarding it, and have tried to interpret the observations of various investigators on this cell cord.

To Carl Semper is given the credit of the discovery of the so-called "lateral line" in annelids. In his extended work on the relationships of segmented animals<sup>1</sup> published in 1876 he described and figured this structure and discussed its probable significance.

He characterized it as a row of cells belonging to the side tract of the worm, lying between the two lateral bands of muscles and extending from the tail, where it originates from the ectoderm, to the front of the head, where it passes into the oesophageal collar. In the case of individuals undergoing fission, the cord forms in the developing head a sensory plate which gives rise to a part of the brain and commissure, and as it seemed to him might even give rise to muscular fibres.

He found the cell cord to exist in *Chaetogaster*, *Nais*, *Tubifex*, and *Psammoryctes*. In a species of the last genus he figures the connection of the cord of cells with the commissure near the brain.<sup>2</sup>

Semper considers that this annelid lateral line may be compared to the lateral line of the lower vertebrates, and thinks that there is a resemblance between it and the nerve of the lateral-line system of fishes and larval amphibians, his reasons being

<sup>1</sup> C. Semper, *Die Verwandtschaftsbeziehungen der gegliederten Thiere*, III, *Strobilation und Segmentation*, *Arbeit. a. d. Zool.-Zoot. Inst. Würzburg*, Bd. III, 1876.

<sup>2</sup> Semper, *l.c.*, Taf. XI, Fig. 3, *sl.*

its origin from the epidermis, its position between the dorsal and ventral muscles, its separation from the epidermis by the pushing in of muscles, and its connection with the oesophageal collar. He thinks that no such physiological importance can be ascribed to it, as in the fishes with its sense organs, but its morphological significance is unmistakable and perhaps greater than has yet appeared.

Semper makes the very interesting observation that the origin of the eyes of *Nais* is confined to the lateral line, and raises the question as to the lateral trunk eyes of *Polyophthalmus* being also limited in their development to the lateral line and its transformations.

Bütschli<sup>1</sup> misinterpreted Semper's observations and supposed that the lateral lines of which he spoke were merely two of the many interruptions in the longitudinal muscle layer.

Eisig<sup>2</sup> discusses the "lateral line" of *Nais* and comes to the conclusion that the homology instituted by Semper between this formation and the lateral line of vertebrates does not exist. His inference from the account of Semper's discovery is that in *Nais* a problematic cell cord occupies a position similar to that occupied by the lateral-line system in vertebrates.

Eisig's reasons for concluding as he does are based on the fact that by the lateral line of vertebrates we mean the whole lateral-line system, and that Semper's cell cord cannot be compared to any one part of the system. Semper's intimation that it is comparable to the lateral nerve is not in harmony with what is known of the development of this organ in vertebrates.

However, considering the fact that Semper found the eyes of *Nais* to occur in this line and thought it probable that the trunk eyes of *Polyophthalmus* would be found similarly situated, we must admit that he had some ground for making the comparison. The "Anlage" of the lateral-line system is a cord of cells which grows down the side of the body and differentiates later into the different organs of the system. Now, if the lateral

<sup>1</sup> O. Bütschli, Untersuchungen über freilebende Nematoden und die Gattung *Chaetonotus*, *Zeit. f. wiss. Zool.*, Bd. XXVI, 1876, p. 401.

<sup>2</sup> Hugo Eisig, Die Seitenorgane und becherförmigen Organe der Capitelliden, *Mitth. a. d. Zool. Sta. Neapel*, Bd. I, 1879, pp. 322-325.

line described by Semper is a cell cord in which sense organs are found, the similarity to the vertebrate "Anlage" is striking, to say the least.

In a preliminary paper Vejdvoský<sup>1</sup> notes the occurrence of a "lateral line" or lateral cord in *Anachaeta*, and thinks it probable that it functions as a sympathetic nerve, and, being connected with the commissure, suggests that it might be called a "vagus nerve." Timm<sup>2</sup> has noted the occurrence of this cell cord in *Phreoryctes*, and Bülow<sup>3</sup> finds it in *Lumbriculus*, and thinks it is undoubtedly nervous in its character. Reighard<sup>4</sup> found the cells in *Dero*, but could not trace the cord as far forward as the commissure. Štolc<sup>5</sup> called it a lateral nerve and thought that it probably arose from the two large cerebral nerves.

Vejdvoský<sup>6</sup> in his valuable monograph considers that this structure is of general occurrence in the Oligochaeta, and proposes that the term ganglion cell cord ("ganglienzellstränge") be applied to it in view of the fact that its homology with the lateral line of vertebrates has not been thoroughly established. He says that the connection of the cord with the commissure is made out with great difficulty. In many worms the cord in the anterior segments is differentiated into fibres which connect with the brain,<sup>7</sup> while in the higher forms the undifferentiated cells merge into the brain or rather into the commissure near the point where it leaves the brain. The cells which make up this cord are mostly unipolar, and only in *Enchytraeidae* did he find bipolar and multipolar cells. Lateral branches, sometimes composed of ganglionic cells and sometimes of fibres, are given off from the main cord.

<sup>1</sup> F. Vejdvoský, Vorläufige Mittheilungen über die fortgesetzten Oligochaetenstudien, *Zool. Anz.*, 1879, p. 184.

<sup>2</sup> R. Timm, Beobachtungen an *Phreoryctes menkeanus* Hoff. und *Nais*, *Arbeit. a. d. Zool.-Zoot. Inst. Würzburg*, Bd. VI, 1883, pp. 131, 132.

<sup>3</sup> C. Bülow, Die Keimschichten des wachsenden Schwanzendes von *Lumbriculus variegatus*, etc., *Zeit. f. wiss. Zool.*, Bd. XXXIX, 1883, p. 75.

<sup>4</sup> J. Reighard, Anatomy and Histology of *Aulophorus vagus*, *Proc. Am. Acad. Arts and Sci.*, Vol. XX, 1884, p. 104.

<sup>5</sup> A. Štolc, *Dero digitata*, etc., p. 91.

<sup>6</sup> F. Vejdvoský, System und Morphologie der Oligochaeten, 1884, pp. 93, 94.

<sup>7</sup> *L.c.*, Taf. II, Fig. 17.

He mentions<sup>1</sup> having found, in *Lumbriculus*, *Rhynchelmis*, and *Claparedilla*, "cup-shaped organs" in the epidermis situated on the ganglion cell cord and in the region of the break in the longitudinal muscle layer. They agree in position with the lateral organs described by Eisig in the capitellids. These organs are in the epidermis, have cilia, are arranged one pair in each segment, and are retractile. I do not understand from his work that they are in the ganglion cell cord, but that they are in the epidermis above it.

In his monograph Eisig<sup>2</sup> affirms his former statement regarding the problematic character of Semper's "lateral line," and cites Vejdovský's statement that it is a sympathetic nerve. He thinks that the "cup-shaped organs" described by Vejdovský if more fully investigated may be found to be homologous to those described by him in Capitellidae.

An entirely different view is held by Hesse,<sup>3</sup> whose investigations have led him to decide that the two lateral lines are composed of the nucleated plasma parts of the circular muscles which are collected into these two lines. His early observations were made on *Nais* and *Fredericia*. In a later work<sup>4</sup> he extends his observations to *Chaetogaster*, *Stylaria*, *Tubifex*, *Limnodrilus*, and *Lumbriculus*, and thinks that this peculiarity is common to all Oligochaetes.

In the excellent monograph of the Oligochaetes recently issued by Beddard,<sup>5</sup> this structure is referred to as the lateral nerve or ganglionic chain, and Vejdovský's interpretation, that it may be compared to the sympathetic system of higher forms, seems to be preferred. However, Hesse's view is stated, without comment, in a footnote.

Summing up briefly these observations, we conclude as follows regarding this structure:

<sup>1</sup> F. Vejdovský, *l.c.*, p. 98.

<sup>2</sup> H. Eisig, *Monographie der Capitelliden des Golfes von Neapel, etc., Fauna u. Flora d. Golfes von Neapel*, 1887, p. 510.

<sup>3</sup> R. Hesse, *Beiträge zur Kenntnis des Baues der Enchytraeiden*, *Zeit. f. wiss. Zool.*, Bd. LVII, 1893, p. 6.

<sup>4</sup> R. Hesse, *Zur vergleichenden Anatomie der Oligochaeten*, *Zeit. f. wiss. Zool.*, Bd. LVIII, 1894, pp. 396, 397, 401, 402.

<sup>5</sup> F. E. Beddard, *Monograph of the Oligochaeta*, Oxford, 1895, pp. 20, 21.



(1) In most, if not in all, Oligochaetes there exists on either side of the body in the lateral line a row of peculiar cells extending the entire length of the worm.

(2) Sense organs may occur in or near this line.

(3) In some forms this cell cord apparently merges into the brain.

(4) In case of multiplication by fission a proliferation of cells either in or near this line takes place.

As to the probable significance of this cell cord the following views have been advanced:

(1) It is a homologue of the lateral-line system of the lower vertebrates (Semper).

(2) It is a part of a sympathetic nervous system (Vejdovský).

(3) It is a problematic cell cord of doubtful significance (Eisig).

(4) It is an aggregation of the nucleated plasma parts of the circular muscle cells (Hesse).

(b) *Descriptive.*

The "lateral line" in *Dero vaga* was noted at the beginning of my work on that form, and I have made a careful study of it in order to determine its true nature. Starting with the idea that we have here a cord of cells which may be compared to the "Anlage" of the lateral-line system of lower vertebrates, I have been led to accept the view advanced by Hesse, and am now confident that it cannot be interpreted as a nervous structure. In this form the "lateral line" occupies the break between the dorsal and ventral half of the lateral muscle areas and extends from the head segment to the growing zone at the tail, where it increases in width and is lost in the embryonic tissue present in that region. The parts of the cells which are clearly visible are elongate pear-shaped and extend horizontally into the coelom, forming a strand composed of two or three rows of nuclei. Pl. XIV, Fig. 18, shows the cells as they appear in a thick ( $15\ \mu$ ) cross-section of the worm. The nerve given off from the ventral cord is the fourth lateral. The muscles extending from the vicinity of the strand to the



alimentary canal form a part of the dissepiment, while those immediately below the strand extend back of the dissepiment, and many of them run to the body wall close to the large sense organ of the lesser band. There is a possibility that these muscles (Fig. 18, *l.l.mus.*) may correspond to the muscles of the lateral organs found by Eisig in the Capitellidae and by Meyer in Polyopthalmus. In the dissected specimen shown in Pl. XIV, Fig. 19, the connection between the nuclei and the muscle fibres may be seen, and also the position of the sense organs relative to the strand. A frontal section is shown in Fig. 20, and a more highly magnified portion of the same in Fig. 21.

(c) *Interpretation of Previous Observations.*

Since the cells forming the "lateral line" are without doubt muscle cells, it seems necessary to attempt an interpretation of the observations made by several workers who held to the nervous character of the structure.

In the first place, all have said that it was in connection with the brain or commissure, but no one has figured a definite connection such as we should expect. Vejdovský finds that the cells may merge into the central nervous system or may be united to it by fine anastomosing fibres. I have been able to trace the cells up to, or forward of, the brain and have found fine muscle fibres extending from the brain to the lateral wall of the body near the "lateral line." Even should a nerve branch be found going to these cells, it would not prove that the cell cord was a nervous structure, but would rather indicate, as suggested by Hesse, the connection of the nervous system with the circular muscles. However, I do think the muscles are not all innervated from the ganglion in the head segment.

Vejdovský's term "ganglion cell" will hardly apply to these cells from what has been shown to be their true nature. Another point opposed to Vejdovský's view is that the cells have their long diameter at right angles to the cord, and not in the line with the cord (Pl. XIV, Fig. 19).

As regards the same author's view that this cell cord represents the sympathetic system, I can find no nerves extending from the line to the alimentary canal, to the setae, and to the nephridial openings, but I do find muscle fibres in these positions (Pl. XIV, Fig. 18), and some of them I at first mistook for nerves, but later was convinced that they were muscles.

In my early investigation I thought I had found sense organs in this line, but later I found the organs in the epidermis just above and below the line. The line of cells also increases in width near the setae and at the dissepiment, but this is due to a massing of muscles in these regions, and not to the presence of sense organs.

Vejdovský also states, in describing the large sense organs of *N. appendiculata*, that the "lateral ganglion cell cords" give off branches encircling the segments, and that the organs are in these bands and are innervated from them, and not from the central nervous system.<sup>1</sup>

In *Dero* there is a band of cells somewhat resembling the cells of the lateral line which encircles the posterior part of each segment (Pl. XIV, Fig. 10), but I have clearly made out that the sense organs do not occur in this band, but occur before and back of it. Gold chloride does not stain this band, and it in all probability is composed of gland cells.

I do not wish to deny that there are any nervous elements in connection with this line, but I do believe that all the elements that have heretofore been considered nervous are not of that character, and this being the case it certainly bears no relation to the lateral-line system of vertebrates.

#### IV. THEORETICAL CONSIDERATIONS.

##### I. ORIGIN OF METAMERISM.

The facts above presented have a very important bearing in support of the colonial theory of the origin of metamerism. Stated briefly, this theory proposes to account for the origin of segmentation in animals by supposing that they arose from unsegmented forms through the process of multiplication by fission.

<sup>1</sup> Vejdovský, *l.c.*, p. 97.

In illustration I may cite the case of *Microstoma*. This is a small Turbellarian which multiplies by means of fission. This unsegmented worm possesses an alimentary canal extending the length of the body, a so-called "brain" with two lateral nerve trunks and well-marked sense organs situated at the anterior end of the animal.

The process of fission is such that the animal may show, according to von Graff, as many as sixteen individuals of varying ages in one chain. These subsequently separate, forming so many complete individuals. Before separation occurs we have a chain of individuals with a common alimentary canal and a nervous system extending the whole length of the chain. Each individual shows one or two pairs of sense organs at its anterior end. The individual mouth openings have not as yet pushed through. Should this temporary condition become permanent, we should have a segmented form resembling in some essential features an annelid worm.

In the different forms of animals in which fission occurs we find several modes of fission. In the form mentioned each individual proliferates continuously. In some forms the proliferation is confined to one individual, while in others each individual in turn takes part in the proliferation. This last mode could be applied to the segmentation of an annelid.

Supposing that annelids arose in this manner, we should expect to find the segments of the body practically homodynamous, with the more perfectly developed and highly specialized segments at the anterior end of the worm.

In *Dero*, as in many other forms, there is in the adult form a marked "cephalization." Apparently the first five segments are formed after the trunk segments are laid down. A study of the embryological development of the worm may clear away this apparent objection to the theory, as Professor Whitman has shown in the case of *Clepsine*, that, while differentiation does not become apparent in the head as early as it does in the trunk, the segments forming the head are really laid down before those of the trunk region.

In support of the theory I have shown that in *Dero* there is a ganglionic swelling and four lateral nerves for each segment

of the body, and, furthermore, that there is a gradual shading off from the first ganglion to those in the middle region of the body. The sense organs I have been able to trace in lines extending the whole length of the worm. Posterior to the fifth segment the glandular bands and dotted grooves occur on every segment. I have not been able to trace them to the first segment. The first five segments contain certain organs which are necessary for the proper maintenance of the life of the worm, and it is not improbable that this differentiation may account for a partial obliteration of the metameric features in this region.

## 2. SEGMENTAL SENSE ORGANS IN ANNELIDS AND ORGANS OF SPECIAL SENSE IN HIGHER ANIMALS.

From the discussion of the so-called "lateral line" in annelids it now appears that there can be no homology between annelids and vertebrates based on this structure. It will be well, however, to call to mind other lateral-line homologies which have been suggested by several writers who take the segmental sense organs as a basis for comparison.

In 1879 Eisig threw doubt on the homology instituted by Semper, and considered that the segmentally arranged sense organs of the Capitellidae were homologous with the sense organs of the lateral-line system found in fishes and larval batrachians.

In 1887 he affirmed his former view and cited in support of his homology the work of Meyer on *Polyopthalmus* and Beard<sup>1</sup> on sense organs of the lateral line in vertebrates.

Professor Whitman in 1884<sup>2</sup> and again in 1889<sup>3</sup> suggested that the segmental sense organs of the leech are identical with the lateral-line organs of the vertebrates. He further suggests "that the segmental sense organs of annelids have formed the

<sup>1</sup> J. Beard, On the Segmental Sense Organs of the Lateral Line, etc., *Zool. Anz.*, Bd. VII, 1884, pp. 123-140.

<sup>2</sup> C. O. Whitman, Segmental Sense Organs of the Leech, *Am. Nat.*, Vol. XVIII, 1884, pp. 1104-1109.

<sup>3</sup> *Ibid.*, Some New Facts about the Hirudinea, *Journ. of Morph.*, Vol. II, 1889, pp. 592-595.



starting-point for the development of the organs of special sense in the higher animals, not excepting even the eyes of vertebrates."<sup>1</sup> He also advocates the view that "the ancestral segmental sense organs were not limited to a single pair of lateral lines, but to several paired lines symmetrically placed on the dorso-lateral and ventro-lateral surface."<sup>2</sup>

Objections to the homology held by the investigators above mentioned have been brought forward by a number of men, including Balfour, who in regard to Capitellidae says: "I am not inclined to think that there is a true homology between these organs and the lateral line of Vertebrata."<sup>3</sup>

It must be admitted that in the adult form there is a notable difference between the systems of organs in annelids and vertebrates. If we consider the annelid organs as the starting-point, we find some points of similarity, such as the metameric arrangement and the general plan of structure of all the organs, which may prove of much importance in the further study of the lateral-line system.

THE UNIVERSITY OF CHICAGO,  
June, 1896.

<sup>1</sup> C. O. Whitman, *Journ. of Morph.*, pp. 592, 593.

<sup>2</sup> *Ibid.*, p. 595.

<sup>3</sup> F. M. Balfour, *Complete Works*, Vol. III, p. 538, note.



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<i>b.v.</i>	blood vessel.	<i>l.s.o.</i>	lateral row of sense organs.
<i>c.mus.</i>	circular muscle layer.	<i>m.</i>	mouth.
<i>com.</i>	commissure.	<i>m.s.o.</i>	median row of sense organs.
<i>d.g.</i>	dorsal ganglion.	<i>m.s.n.</i>	main sympathetic nerve.
<i>d.gr.</i>	dotted groove.	<i>mus.</i>	muscle.
<i>dis.</i>	dissepiment.	<i>n.<sup>1</sup></i>	first nerve from dorsal ganglion.
<i>d-l.s.o.</i>	dorso-lateral row of sense organs.	<i>n.<sup>2</sup></i>	second nerve from dorsal ganglion.
<i>d.s.</i>	dorsal setae.	<i>n.<sup>3</sup></i>	third nerve from dorsal ganglion.
<i>ep.</i>	epidermis.	<i>n.<sup>4</sup></i>	fourth nerve from dorsal ganglion.
<i>fb.</i>	fibrous part of ventral cord.	<i>n.o.</i>	nephridial opening.
<i>g.c.</i>	ganglion cell.	<i>p.p.c.mus.</i>	plasma part of circular muscles.
<i>gl.b.</i>	glandular band.	<i>ph.</i>	pharynx.
<i>gf.</i>	giant fibres.	<i>s.c.</i>	sense cell.
<i>i.n.</i>	intermediary nerve.	<i>s.g.</i>	sympathetic ganglion.
<i>l.l.</i>	lateral line.	<i>s.n.</i>	sympathetic nerve.
<i>l.l.mus.</i>	lateral line muscle.	<i>s.o.</i>	sense organ.
<i>l.mus.</i>	longitudinal muscle layer.	<i>s.s.</i>	setae sac.
<i>l.n.</i>	lateral nerve.	<i>v.c.</i>	ventral cord.
<i>l.n.<sup>1</sup></i>	first lateral nerve from ventral ganglion.	<i>v.g.</i>	ventral ganglion.
<i>l.n.<sup>2</sup></i>	second lateral nerve from ventral ganglion.	<i>v-l.s.o.</i>	ventro-lateral row of sense organs.
<i>l.n.<sup>3</sup></i>	third lateral nerve from ventral ganglion.	<i>v.s.</i>	ventral setae.
<i>l.n.<sup>4</sup></i>	fourth lateral nerve from ventral ganglion.		

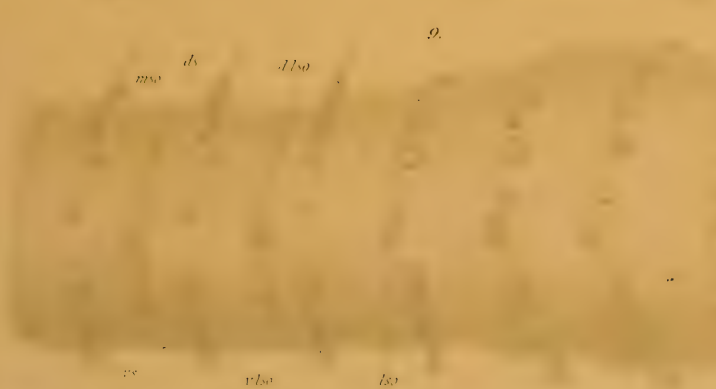
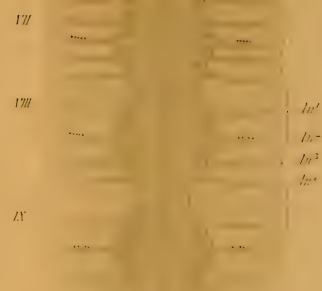
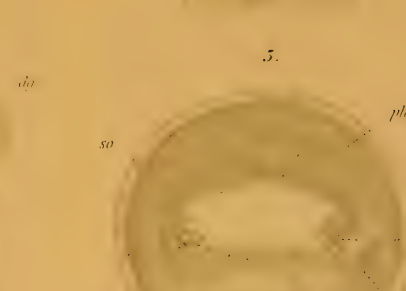
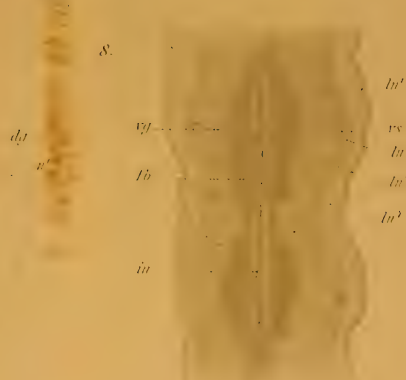
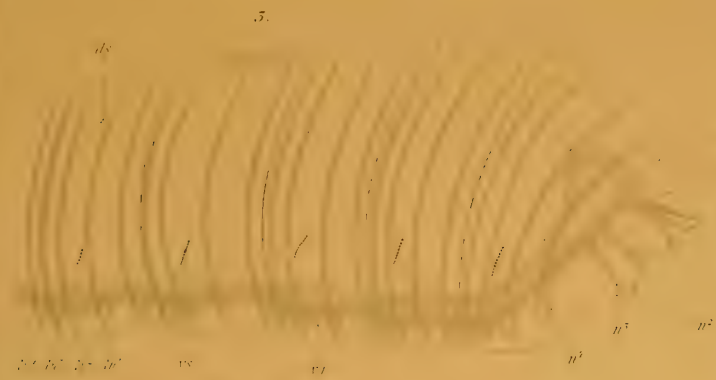
## EXPLANATION OF PLATE XIII.

- FIG. 1. *Dero vaga*, worm in case.  
FIG. 2. Branchial apparatus at posterior end of body.  
FIG. 3. Lateral view, showing central nervous system and lateral nerves.  
FIG. 4. Lateral view of sympathetic system—outer coat of pharynx removed.  
FIG. 5. Cross section of worm through pharynx. Gold chloride preparation, showing sympathetic and lateral nerves.  
FIG. 6. Dorsal view of nervous system in anterior segments.  
FIG. 7. Dorsal view of nervous system at growing zone.  
FIG. 8. Dorsal view of frontal section of nerve cord in one segment. Gold chloride preparation.  
FIG. 9. Lateral view of anterior portion of a worm, showing sense organs.













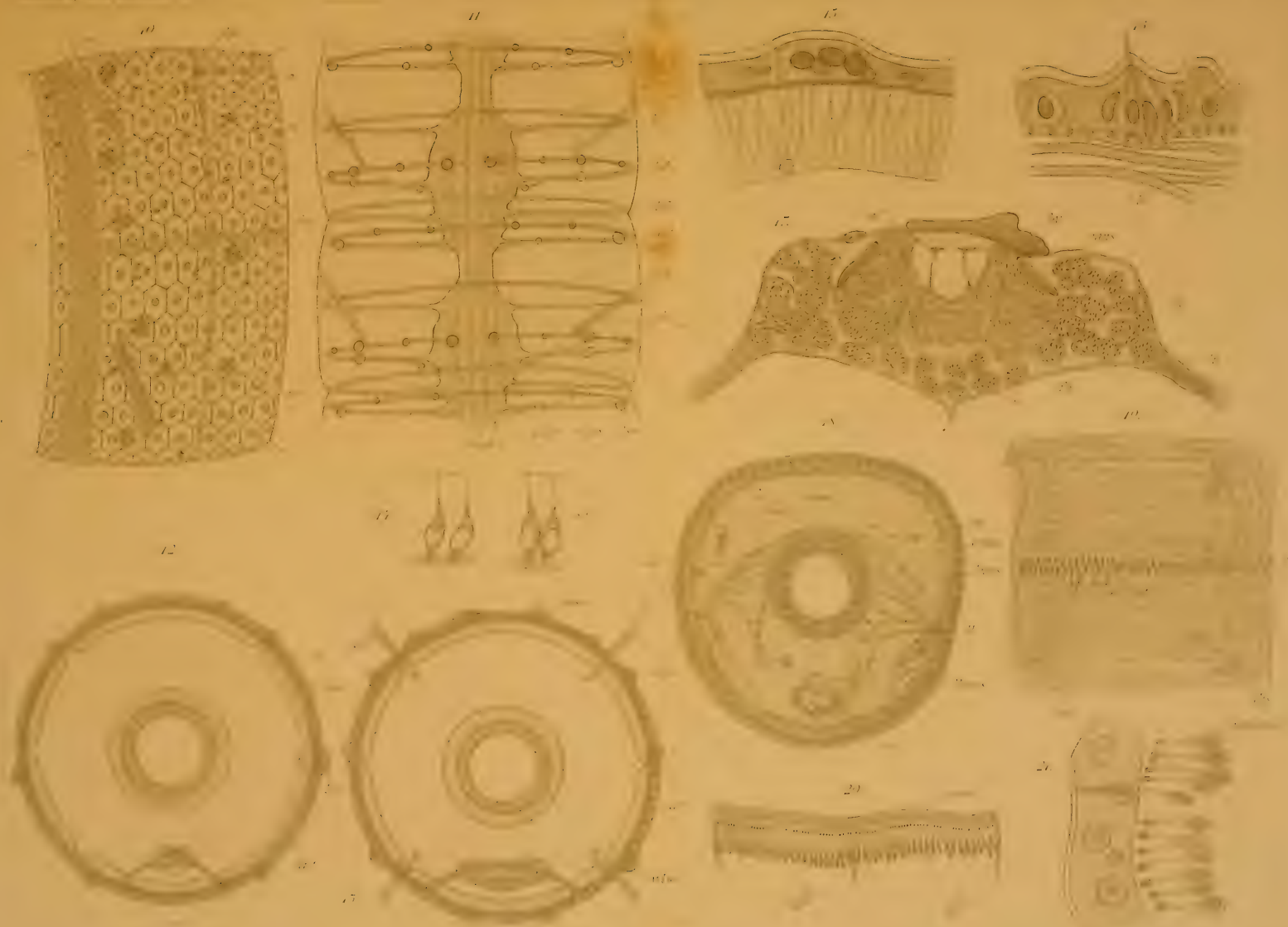
## EXPLANATION OF PLATE XIV.

- FIG. 10. Lateral view of one segment much enlarged.
- FIG. 11. Diagram showing connection of nervous system and sense organs.
- FIG. 12. Diagram of cross section of worm through the lesser band of sense organs.
- FIG. 13. Diagram of cross section of worm through greater band of sense organs.
- FIG. 14. Sense cells. Gold chloride preparation. ( $\times 2000$  reduced  $\frac{1}{2}$ .)
- FIG. 15. Sense organ seen in cross section of worm prepared with acetic corrosive sublimate. ( $\times 2000$  reduced  $\frac{1}{2}$ .)
- FIG. 16. Sense organ as seen in frontal section of worm prepared with Hermann's fluid. ( $\times 2000$  reduced  $\frac{1}{2}$ .)
- FIG. 17. Cross section of ventral cord. ( $\times 2000$  reduced  $\frac{1}{2}$ .)
- FIG. 18. Cross section of a worm near dissepiment.
- FIG. 19. One segment dissected to show "lateral line."
- FIG. 20. Frontal section through "lateral line." ( $\times 400$  reduced  $\frac{1}{2}$ .)
- FIG. 21. Portion of same more highly magnified. ( $\times 2000$  reduced  $\frac{1}{2}$ .)













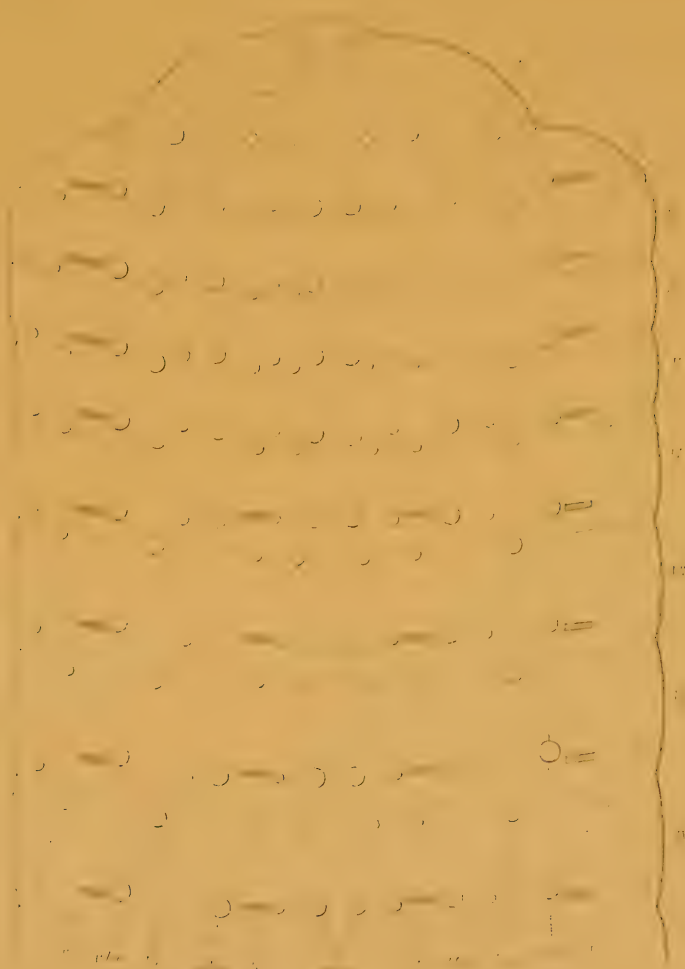


## EXPLANATION OF PLATE XV.

Diagram showing anterior segments of a worm cut on ventral side and spread out to show arrangement of sense organs.











# THE ORIGIN AND BEHAVIOR OF THE CENTRO- SOMES IN THE ANNELID EGG.

A. D. MEAD.

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## I. GENERAL.

No protoplasmic structure has been the object of greater interest or incited more investigation during the last few years than the centrosome. The fact that it remained undiscovered until a comparatively recent date, and that, with modern technique, it has been found to occur in cells representing many different tissues in a variety of animals and plants, would alone place the centrosome in a conspicuous position in the literature on the cell; but the difficulty and uncertainty often attending the demonstration of this structure, its minuteness, the variety of phases under which it manifests itself, the diversity and supreme importance of the functions attributed to it, either as the bearer of hereditary qualities or as the organizer and director of cell-activities, have stimulated research and discussion to an extraordinary degree.

The literature on the centrosome has developed two general problems toward the solution of which the results of these observations are contributed.

(a) *The Morphological Relation of the Centrosome to the Other Organs of the Cell.*

This problem demands consideration of the doubt as to the very *existence* of the centrosome as a definite cell-organ, and of the uncertainty as to the *identity* of this organ in case it exists.

The possibility that bodies described as centrosomes are frequently artifacts does not rest solely upon the negative evidence of numerous observers who fail to find centrosomes where they might be expected to occur, but upon the fact that similar bodies can be produced by the coagulative action of certain reagents.

The classic centrosomes, which dance the quadrille in the egg of *Strongylocentrotus lividus* (Fol, '91) are not found by later workers in the eggs of closely related sea-urchins. In his first paper on the "Fertilization of *Toxopneustes*," Wilson maintains that in well-preserved material "there is absolutely nothing to be identified as a centrosome," though irregular clumps—fortuitous groups of granules—closely similar to those described by Fol as "centrosomes" may be produced by the destructive action of picro-osmic acid. Eismond (*Anat. Anz.*, X, 7, 8) and others have suggested that the centrosomes or "centrioles" have been, at least in some cases, produced by the clotting action of reagents, and do not represent actual cell-organs. But in view of the fact that so many investigators have demonstrated the centrosome in many different cells and with different reagents, and that in some instances this structure can be followed through a constant and continuous series of changes, including growth and division, it is safe to maintain that the centrosome is an actual organ of the cell.

While the existence of the centrosome has been established beyond a reasonable doubt, there still remains the puzzling question of *identity*. Structures widely different in appearance have been called centrosomes, and the same structure has been designated by this and by various other names. Much confusion, therefore, arises from the terminology, though the discrepancies can by no means be assigned to this cause alone, for there are differences of interpretation in regard to the morphological limitations of the structure itself.

Watasé's endeavor to bring into relation the various protoplasmic structures, cyto-microsomes, "centrosomes," *Zwischenkörper*, rod-like "centrosomes" of the pigment-cell, contraction-band of the muscle-cell etc., is an attempt to extend, not the terminology, but the homology of the centrosome. Which of the three concentric structures — centrosphere, centrosome, or centriole — at the centre of the aster in *Echinus* (Boveri) is the morphological equivalent of the centrosomes of *Strongylocentrotus* (Fol), the large centrosphere of *Toxopneustes* (Wilson), the scattered centrosomes in the leucocyte (Heidenhain), the centrosphere-like centrosomes of *Crepidula* (Conklin), the minute centrosomes of *Myzostoma* (Wheeler), *Chætopterus* (Mead), and *Thalassema* (Griffin), must, terminology aside, remain a matter of interpretation based on structural and physiological characters.

It is usually assumed that that structure in the aster which persists through the various stages of mitosis is to be considered the centrosome, whether it be a large reticular area (*Crepidula*), a minute dot frequently surrounded by such an area, or a structure midway between the two (*Echinus*). On this principle Wilson, in his paper on the "Archoplasm, Centrosome, and Chromatin of the Sea-urchin Egg," raises his previously described "central mass" of the aster of *Toxopneustes* to the morphological value of a centrosome, though he has since, on other grounds, altered this interpretation. "What, then, shall we identify in the sperm-aster of *Toxopneustes* as the 'centrosome' in Boveri's sense, *i.e.*, as the structure that divides to form the dynamic centres of the ensuing cleavage? I think the only structure that can answer to this definition is the central mass of the aster, *i.e.*, the substance of the original middle-piece, without regard to its subsequent morphological differentiation."

It was on precisely these grounds that, in a preliminary paper on the "Fecundation of *Chætopterus*," I identified the minute dark granules in the centres of the asters as centrosomes, and endeavored to show that the centrosomes which were developed in connection with the sperm-aster persist and, by successive divisions, furnish the centrosomes of each cleavage-spindle up

to the 8-cell stage. Having requested some of my preparations for examination, Wilson says in referring to them (*Atlas of Fertilization*, p. 20): "The central mass of the aster undoubtedly contains at this period (early phase of the cleavage-amphiaster) one or two deeply staining centrioles, which in this case may possibly have the morphological value of centrosomes." However, reëxamination of all the stages with new material supports my earlier interpretation that these structures have unquestionably the morphological value of centrosomes.

More recently Wilson also has discovered two deeply staining "centrioles" in the aster of the egg of the annelid *Nereis*, and Griffin, working under his direction, has found the same in *Thalassema*. Griffin has also showed that these granules, arising in the sperm-aster, persist through the first cleavage-amphiaster, divide and give rise to the centrosomes of the succeeding amphiassters. In view of these results, which indicate that "the true centrosome certainly corresponds to the central granule or centriole," Wilson is inclined to modify again his interpretation of the identity of the centrosome, and to believe that further research will bring out the "minute central centrosome" in *Toxopneustes*, and in all similar cases where they appear to be absent.

I am indebted to Dr. F. R. Lillie for permission to refer to some extremely interesting unpublished observations on the egg-centrosomes of *Unio* which, doubtless, will throw a great deal of light upon the question of *identity*. From the stage in which it has the appearance of a minute, deeply staining dot, the centrosome is traced, step by step, through a metamorphosis in many of whose phases its identity would not ordinarily be recognized.

The problem of the morphological relation of the centrosome to the other organs of the cell involves also the important questions of its origin and its persistence. This question, both sides of which have the support of eminent authority, has been stated by Watasé ('94) in the following terms: "According to the one view (1) the centrosome is a permanent or ultimate organ of the cell, an organ *sui generis*, and coexistent with other organs



of the cell, as the nucleus and the cytoplasm. According to the other view (2) the centrosome is a derivative structure, arising by the modification of some preëxisting element in the cell, as the chromosome, nucleolus, or the cytoplasm."

The theory that the centrosome is a permanent and ultimate organ of the cell finds direct support in the observations of a number of investigators, which prove beyond doubt that the centrosome is capable of self-division and growth, and that it may persist from one generation to another.

In *Chætopterus* the centrosomes can be seen distinctly in every phase of mitosis, and are always surrounded by an aster, even in the resting stage. On the other hand, in certain animals, the centrosomes are not always demonstrable in the asters. Sometimes both the asters and the centrosomes vanish for a while and then reappear (Wheeler, Lillie, MacFarland, etc.). It is conceivable, however, that, though invisible, the centrosomes may be present in the cell, for they have been found to lie naked in the cytoplasm, bereft of rays (Heidenhain, '93).

To maintain that the centrosomes are absent, simply because they are not demonstrable, is, of course, to base an assumption upon negative evidence, a procedure especially dangerous when applied to the centrosome, inasmuch as this structure is, at best, very minute, and comparatively difficult to demonstrate, even when its exact position is indicated by the presence of an aster.

But, admitting that the centrosome is "a persistent morphological element having the power of growth, division, and persistence in the daughter cells," and even admitting that it exists incognito, it remains to be proved that it arises only by division from a preëxisting centrosome and that it does not cease to exist as a morphological element in cells which subsequently possess centrosomes.

Watasé has pointed out that the transition between centrosomes and other structures of the cytoplasm should be sought, not among the most highly developed typical centrosomes, such as are to be found in mitosis, but among structures — centrosomes or homologues of centrosomes — which are less persistent



and pass more easily from one phase into another; for example, the *Zwischenkörper* and the contraction-band in muscle-cells.

But the origin and behavior of the asters and centrosomes in the maturation of *Chætopterus* directly support the interpretation that the centrosomes arise *de novo* out of the cytoplasm and are resolved into it, and that they are not, therefore, permanent organs of the cell, like the chromosomes, although some of them show a considerable degree of persistence.

Leading up to the formation of the first maturation-amphister, there are formed within the egg a large number of distinct asters (seventy-five more or less), two of which — the primary — come to lie at the poles of the maturation-spindle.<sup>1</sup> The view held by the majority of recent workers that the aster is "formed under the influence of the centrosome," or the admission that the centrosome is in any way a constant feature or necessary adjunct of the aster (and without this hypothesis there is no force in the permanent-organ theory) offers a dilemma: either (*a*) this cell (the oöcyte of the first order) contains a very large number of centrosomes which have arisen by the division of a preceding centrosome, or (*b*) it contains a large number of centrosomes which have arisen *de novo* out of the cytoplasm. If one accepts the first alternative, he must imagine a satisfactory explanation of the origin of the many centrosomes from a preceding element of the same kind, and of their distribution to the various portions of the cytoplasm, and must also account for the total disappearance of by far the larger portion of them. It is hard to reconcile such wholesale disappearance with the notion of the centrosome as a permanent and ultimate organ of the cell.

Multiple asters, similar to those in *Chætopterus*, have been described by Carnoy in *Ascaris* during the formation of the second polar globule, and by Reinke ('94) in the peritoneal cells of the larval salamander. Reinke groups the multiple asters into three classes, — primary, secondary, and tertiary mechanical centres, — according to the degree of their development. The primary mechanical centres, which contain a true centrosome, arise by the coalescence and further development

<sup>1</sup> Possibly several coalesce to form each aster (see p. 195).

of the secondary and these in turn arise in a similar manner from the tertiary centres. Watasé has seen in the egg of *Macrobdella* "a series of thirteen asters, ranging from a miniature aster, with a microsome for its centre, to the normal aster, with a veritable centrosome."

Morgan ('96) found numerous "artificial astrospheres" in sea-urchin eggs kept in sea-water to which  $1\frac{1}{2}\%$  salt had been added. The salt, he believes, stimulates the living eggs to produce these structures. It is interesting that the multiple asters appear in the eggs of *Chætopterus* immediately after they have been deposited in sea-water. The latter probably contains more salt than the fluids of the body-cavity of the worm.

R. Hertwig ('95) has shown that minute quantities of strichnine stimulate the production of asters (even amphiasters) in the matured unfertilized egg of the sea-urchin. No centrosomes, however, were found.

Osterhout ('97) has recently described in *Equisetum* the origin of the amphiaster from multiple asters, though no centrosomes were demonstrable. In the egg of *Unio*, during the metaphase of the second maturation-spindle, a supernumerary aster appears, remains for a short period, and then vanishes. The interesting question presents itself,—If this supernumerary aster contains a centrosome, from what preceding centrosome does it arise? If it does not contain a centrosome, the latter is not a necessary feature, much less the originator, of the aster.

Referring to the observations of Reinke, Watasé, Morgan, and Hertwig, Wilson says: "All these observations are of high interest in their bearing on the historical origin of the centrosome; but they do not prove that the centrosome of the normal aster ever arises by free formation. On the whole, the evidence has steadily increased that the centrosome is to be classed among the permanent cell-organs; but whether it ranks with the nucleus in this regard must be left an open question" ("Cell," p. 226).<sup>1</sup>

<sup>1</sup> In the appendix to the second edition of this book on the "Cell," Wilson is inclined to adopt a conclusion nearly related to that which he maintained in an earlier paper ('95), by reason of recent observations which "throw grave doubts upon the hypothesis of the universal autonomy and genetic continuity of the centrosome."

The multiple asters in the egg of *Chætopterus* are certainly normal; they are demonstrable in the *living eggs*, are brought out by various reagents, are a constant feature of every egg at a certain stage of its development, and always undergo a constant and continuous series of consecutive changes. Therefore, as far as the phenomena in *Chætopterus* prove the free formation of the centrosome at all, they prove it in the normal aster; and the same can be said of Reinke's observation.

(b) *The Function of the Centrosomes in Fertilization.*

The classic papers of Boveri ('87, '91) and Fol ('91) formulated and brought into prominence two distinct theories of fertilization: that of Boveri rests upon the supposition that the centrosome is the dynamic centre of the cell and initiates cell-activities. It implies also that the centrosome is a permanent and ultimate cell-organ, handed down from one generation to another by means of the spermatozoön. The gist of the theory appears in a paragraph from his earlier paper: "The ripe eggs possess all the organs and qualities necessary for division, excepting the centrosome, by which division is initiated. The spermatozoön, on the other hand, is provided with a centrosome, but lacks the substance in which this organ of division may exert its activity. Through the union of the two cells in fertilization, all the essential organs necessary for division are brought together; the egg now contains a centrosome which, by its own division, leads the way in the embryonic development." He writes also: "The end of fertilization is the union of the germ nuclei and the equal distribution of their substance, while the active agent in this process is the centrosome. . . . It is the centrosome alone that causes the division of the egg, and is, therefore, the fertilizing element proper." To these conclusions Wilson subscribes.<sup>1</sup>

Fol maintained, on the other hand, that, in fertilization, the centrosomes of the cleavage-amphiaster arise by the fusion of sperm-centrosomes and egg-centrosomes, just as the cleavage-nucleus is formed by the union of the sperm-nucleus and the egg-nucleus. The centrosome at either pole of the amphiaster

<sup>1</sup> Wilson, "The Cell," p. 140.

is composed of both male and female elements; for the original sperm-centrosome and egg-centrosome divide into two, and each daughter-centrosome derived from the sperm fuses with one of the daughter-centrosomes derived from the egg. The obvious inference which has been drawn from these phenomena is that "fertilization consists, not only in the adding together of the two pronuclei derived from individuals of different sexes, but also in the fusion of four half-centres derived from the father and the mother into two new bodies, the astrocentres" (centrosomes).<sup>1</sup> Obviously, if the permanent presence and fusion of the sperm- and egg-nuclei indicate that these structures are the vehicles of hereditary properties, the same function may be predicated of the centrosomes.

The results of Guignard's researches on the fertilization of the lily and Conklin's work on the gasteropod *Crepidula* agree in all essential respects with those of Fol on the sea-urchin. According to almost every other observer, however, either the sperm-centrosome or the egg-centrosome, or both, disappear before the formation of the cleavage-amphaster—a phenomenon manifestly incompatible with Fol's interpretation of their functions in fertilization. The fusion of the sperm- and egg-centrosomes in fertilization is not of universal occurrence, and the inference that these structures are vehicles for the conveyance of hereditary qualities is unwarranted.

But the observations which discredit Fol's interpretation do not confirm, in every case, Boveri's theory of fertilization. Wheeler showed that in *Myzostoma* there is no indication that a centrosome is brought in by the spermatozoon or that a centrosome or aster subsequently develops in connection with the sperm-nucleus. On the other hand, the egg-centrosomes derived from the second maturation-spindle accompany the egg-pronucleus as it approaches that of the sperm. During the approach of the pronuclei the egg-centrosomes move away from each other as though they were to form the poles of the cleavage-amphaster; but during a certain brief period "it is extremely difficult or even impossible to make out the egg-centrosomes." It is probable that these bodies—certainly not

<sup>1</sup> Fol ('91), p. 274; Conklin ('94), p. 18.



the sperm-centrosomes — enter into the formation of the first cleavage-spindle.

Miss Foot's ('97) account of the origin of the cleavage-centrosomes in *Allolobophora fatida* does not accord with Boveri's theory of fertilization nor with that of Fol. "The egg attraction sphere is present during the two maturation divisions, but after the second polar body is formed and the female pronucleus begins to develop it totally disappears. The sperm attraction sphere is present until the head of the spermatozoön begins to develop into the male pronucleus, when it also totally disappears. Both spheres are absent during a relatively long period (*i.e.*, while the growing pronuclei are developing); and when the two pronuclei have attained their maximum size and are in contact, two attraction spheres again appear in the cytoplasm and the cleavage-spindle is formed."

Lillie ('97) has observed a peculiar behavior of the centrosomes and asters in *Unio*. After undergoing extraordinary metamorphoses, the egg-centrosomes alone enter into the formation of the cleavage-amphiasma, as they do in *Myzostoma*; a conspicuous comet-like aster with a centrosome develops in connection with the sperm-nucleus, but totally disappears before the pronuclei come together.

MacFarland's ('97) results on *Pleurophyllidia* are similar to those of Miss Foot on *Allolobophora* in that both egg- and sperm-centres are apparently absent during a certain period preceding the union of the pronuclei.

All these observations form a serious obstacle in the way of accepting Boveri's theory of fertilization, but there is a further and perhaps more serious difficulty in the insecurity of the hypotheses underlying the theory itself; *viz.*, (*a*) that the centrosome is a permanent organ of the cell, and (*b*) that it initiates and directs the cell-activities. We have already referred to the question of the validity of the first of these hypotheses (p. 185), and comparison of the normal fertilization phenomena of various forms leaves room for doubt as to the validity of the second. The eggs of various animals attain to different stages of maturation before fertilization takes place; some remain in the germinal-vesicle stage (with no visible centrosome or aster)



and await the entrance of the spermatozoön (Nereis), some remain in the metaphase of the first maturation-amphiaser (Chætopterus), while others proceed with the formation of the polar globules and the reconstitution of the egg-nucleus before fertilization (sea-urchin). If it is the function of the centrosomes upon being brought into the egg "to organize the machinery of mitotic division,"<sup>1</sup> its task must be very different in different eggs; for in one it must first organize the machinery for the two maturation-divisions, in another it finds this machinery already organized but in a state of rest, and in a third it has to organize only the machinery for the first cleavage-mitosis.

In Chætopterus the behavior of the sperm-centrosomes is strictly orthodox, according to Boveri's doctrine; they are the centrosomes of the first cleavage-spindle, and give rise by division to those of the succeeding spindles, while the egg-centrosomes totally disappear. Nevertheless, it does not seem necessary to conclude that it is by virtue of the presence of the sperm-centrosomes, rather than of the sperm-nucleus, that the maturation-processes are resumed, even if we grant that the spermatozoön brings in the centrosomes. An adequate demonstration that the sperm-centrosomes are actually carried into the egg by the entering sperm is, moreover, extremely difficult to make, and it is perfectly possible, as Miss Foot has urged, that in many cases these structures arise *de novo* out of the egg-cytoplasm in the *vicinity* of the middle-piece of the spermatozoön.

The foregoing considerations lead to the conclusion that the centrosomes in fertilization are neither vehicles of hereditary qualities nor the active agents which organize the machinery of mitotic division, but that they may be, like other centrosomes, "the expression rather than the cause of cell-activities."

<sup>1</sup> Wilson, "The Cell," p. 171.

## II. DESCRIPTIVE.

(a) *Collection and Preparation of Material.*

The observations recorded in the following pages were made upon the eggs of *Chaetopterus pergamentaceus* Cuvier, procured at the Marine Biological Laboratory, Woods Holl, Mass., during the summers of 1894, 1896, and 1897.

These extraordinary annelids are found below low-water mark in leathery tubes. The latter are U-shaped, ten to fifteen inches long, about an inch in diameter in the widest part, and are buried beneath the mud except half an inch at either end. After being removed from the tubes the animals may be kept alive for a few days in an aquarium; they are quite helpless in their new environment, and usually lie on their side, keeping up continuously the rhythmical respiratory movement of their wing-like body-folds, which, under natural conditions, would serve to create currents of water through the tubes. When disturbed at night, they emit a phosphorescent light, apparently dependent upon a secretion from epidermal glands, for the water in which they have been kept becomes itself slightly phosphorescent.

The sexes are readily distinguished, the body-wall being nearly transparent and the posterior segments distended with eggs or spermatozoa. The large parapodia hold the sexual products, and any number may be cut off without injury to the worm. The eggs may be fertilized at any time during the day or night and will develop normally, provided the sperm is added within a few hours after they have been removed from the ovaries. This is true of every individual collected during the months of July and August, and indicates that the eggs are probably carried in the body for many days after they are perfectly mature and ready to be fertilized. If the eggs are kept in sea-water for half an hour or more and not fertilized, all except the smaller ovarian eggs are found to have the first maturation-spindle well formed, in its definitive position, and always in the same stage of development, *i.e.*, the metaphase or equatorial-plate stage. But, if the eggs are ex-

amined after having remained *only a few minutes* in sea-water, they are all found to contain the germinal vesicle and no spindle. It is evident, therefore, that sea-water in some way stimulates the eggs to the production of the maturation-spindles.

(b) *Method of Fixing and Staining.*

The best preparations were obtained by fixing the eggs in Boveri's picro-acetic acid, and staining with Heidenhain's iron-alum hæmatoxylin, followed by orange G. The slides were left in 4% iron-alum for half an hour, rinsed, and left in  $\frac{1}{2}\%$  hæmatoxylin for twelve hours. After drawing the color with iron-alum, the slides were dipped in an aqueous solution of orange G or Bordeaux red. Hermann's fluid, Flemming's fluid, and a mixture of Hermann and formalin also gave satisfactory results, though the staining was not so brilliant. Sublimate-acetic usually wrought havoc in the region of the centrosphere, though the astral rays were not destroyed. The sections varied in thickness from three to seven and one-half microns.

(c) *Origin of the Maturation-Spindle.*

The figures in Plate I represent sections of the unfertilized eggs during the growing-period and up to the formation of the first maturation-spindle. As may be seen in Fig. 1, the smaller eggs lie nearest the lumen of the ovarian tubule. They are characterized by their relatively large nuclei and their compact cytoplasm which stains, throughout the egg, a nearly uniform deep purple (Pl. XVI, Figs. 1, *a*, *b*, and 2).

The older eggs, usually more remote from the lumen of the tubule, are larger, the increase in size being due in great measure to the accumulation of yolk, the distribution of which is accompanied by noteworthy changes in the appearance of the cytoplasm. The latter takes on the appearance of a reticulum composed of beaded strands which stain purple, while within the meshes lie the pale yellow yolk-granules. Up to the time when the egg has attained about two-thirds its full size, only a part of the protoplasm presents the loose reticular appearance; the rest remains as dark purple masses, which I

consider to be equivalent to the *nebenkerne* or paranuclei of various authors (Fig. 1, *c, d, f*). These masses are not homogeneous, but resolve themselves into a cytoplasmic network, of which the meshes are much compressed, and the strands usually parallel with the surface of the nucleus, though at the periphery of the masses they fray out and become continuous with the open network which contains the yolk (Fig. 1, *g*, Fig. 3, *par.n.*, Fig. 4, *par.n.*). Frequently sections show but one mass of this sort, crescentic in outline, with the concavity toward the nucleus, and occasionally some of the constituent fibres, instead of running parallel with the nucleus, are rolled up spirally. As a rule, however, the paranucleus is fragmented and numerous portions are found in a zone between the nucleus and the periphery of the egg. The substance within the meshes of the paranuclear reticulum does not take the yellow, but the blue or the purple stain.

As the egg accumulates more yolk and increases in size, the paranuclei, through a process of continuous ravelling, become resolved into the network which now presents a nearly uniform appearance throughout the cytoplasm. The last traces of the fragmented paranucleus are recognizable even when composed of only two or three strands (Fig. 4). The reticulum can be traced with ease to the extreme periphery, where it forms what, in section, appears to be a distinct beaded line, running entirely around the egg. Immediately inside this outer "pellicle" is a narrow zone containing a single layer of yolk-granules regularly arranged (Fig. 9), and in this zone the strands of the cytoplasm are comparatively few. The nuclear membrane is continuous with the cytoreticulum and presents a similar granular appearance. During the growth of the ovum the character of the reticulum is constantly changing in respect both to the shape of the meshes and to the thickness of the component strands. In later phases of the paranucleus, and immediately after its disappearance, the reticulum is particularly easy to demonstrate. The strands are, at this time, thick, richly stained, and seem to be composed of a series of granules arranged in linear order, while the meshes are small and, in section, nearly circular in outline.



But although the structure of the reticulum, the peripheral egg membrane (pellicle), and the nuclear membrane are easily demonstrable and beautifully clear throughout the cytoplasm, there is, as yet, *no trace of anything suggesting a centrosome or an aster*. As the egg grows larger, however, the outlines of the meshes become polygonal rather than circular (Fig. 6), and show rather pronounced nodes. Eggs which have reached this stage of development, when placed in sea-water continue to develop as far as the formation of the first maturation-spindle. The tendency of the fibrils of the network to straighten becomes accentuated, so that many of them extend in straight lines for a distance several times the diameter of the single meshes (Fig. 6). Moreover, these longer fibrils radiate from common centres, and in this way there arise in the cytoplasm a number of *miniature asters* (Figs. 6, 7). At first the asters possess only two or three rays, but the latter soon increase in number and in length at the direct expense of the remaining network. The formation of asters continues until a climax is reached, when one can count no less than seventy-five distinct asters scattered about through the cytoplasm of a single egg. They are most numerous in the zone formerly occupied by the paranucleus. These structures correspond closely to the "secondary mechanical centres" of Reinke, and, for reasons which appear further on, I have called them *secondary asters*.<sup>1</sup>

All stages in the development of the asters out of the polygonal network may be represented in a single section, yet often many of the larger asters are approximately equal in size, and, though distinct from one another, are frequently so close together that their rays intercross (Fig. 7). The nuclear membrane now presents a peculiar appearance, being drawn out into numerous sharp points—a phenomenon which is probably correlated with the development of the multiple asters (Fig. 7).

The period of development characterized by the multiple asters is not of long duration. Two of the asters gain pre-dominance over the others in point of size, and continue to grow larger, while the others gradually evanesce (Fig. 8).

<sup>1</sup> Mead ('97a).



These two primary asters ("primary mechanical centres," Reinke, '94) arise at some distance from the wall of the germinal vesicle, and usually about ninety degrees from each other, though they may be nearer together or even farther apart. I am not prepared to say at present whether the primary asters are formed by the further growth and specialization of two of the secondary asters or by the union and coalescence of several.

The nuclear membrane regains its regular contour when the multiple asters have vanished, except for a deep sinus in the vicinity of each of the two primary asters (Figs. 8, 9). A well-defined centrosome, staining dark brown, now appears in the centre of each aster, surrounded by an area of lighter color (centrosphere) from which the large granular astral rays diverge in all directions. *These are the centrosomes and asters of the first maturation-spindle* (Figs. 9-13). The centrosphere always stains brown, though very much lighter than the centrosome. The rays from the two asters give the appearance of actually pushing in the nuclear membrane, though the latter remains for a while intact.

The rays of the two primary asters are many of them coarse and quite extensive (Fig. 9). They do not, however, reach the periphery, but break up into the network which extends throughout the whole cytoplasmic portion of the egg. Eventually the nuclear membrane disappears, though the region corresponding to the germinal vesicle still takes a stain different from the rest of the protoplasm and has also a different texture (Figs. 10, 11).

Between the two asters a spindle is formed which remains for some time near the vanishing germinal vesicle, but at right angles to the radius of the egg. The rays from the asters enter the region of the vesicle, the chromosomes gather at the equator of the spindle, and the latter gradually swings around to its definitive position, perpendicular to the surface of the egg (Figs. 10-13). The nucleolus, meantime, breaks up into a number of pieces which remain for a time in the vicinity of the spindle, but gradually degenerate and disappear. The centrosomes divide very early before the spindle begins to move

toward the surface, and appear as two distinct dots in the midst of a clear yellow centrosphere at either end of the spindle.

The fibres of the central spindle differ in color from the other fibres of the asters, staining yellowish brown with orange G (or red with Bordeaux) much like the centrospheres, while the other rays are purplish. The chromosomes, moreover, do not, at first, lie directly between the two poles, but upon the surface of the central spindle (Fig. 10); later they seem to insinuate themselves between its fibres (Fig. 11).

The remains of the germinal vesicle are evident for some time after the spindle has assumed its definitive position, but gradually fade away (Figs. 12-15). Having reached the metaphase the spindle remains without apparent change until the egg is fertilized. The astral rays extend for a long distance into the cytoplasm, intercross with one another, and break up at the ends into the cytotreticulum, which is now much more delicate and finer than during the earlier stages when the paranucleus was disappearing.

It is difficult to understand why the process of karyokinesis should be suspended at this time, for the apparatus of division is apparently ready, the asters are well developed, the chromosomes in position at the equator of the spindle, and the centrosomes have divided in anticipation of the next mitosis (Figs. 13-16).

(d) *Observations upon the Unfertilized Living Eggs.*

During the past summer I have examined a large number of living eggs to confirm the results obtained from the study of preserved material. Full-grown eggs taken from the body-cavity of the female and examined under slight pressure invariably show a germinal vesicle with even contour, and inside the vesicle the nucleolus. The cytoplasm is, at first, uniformly opaque, but in two or three minutes a number of light points appear, and soon the whole cytoplasm is studded with a multitude of *secondary asters*. Since the yolk is repelled from the centre of the asters, the latter appear as transparent spots in an opaque field. After about four minutes two of the asters (primary asters) become especially distinct and the others

gradually evanesce. The two arise separately, sometimes near together, sometimes far apart.

When one of these living primary asters is examined under a high power, the yolk-granules can be seen to move away from the centre with a trembling, vibratory motion. Later the areas free from yolk extend to the region between the two asters, which represents the central spindle, and within about ten minutes after the egg is placed in sea-water the whole amphiaster migrates to its definitive position at the periphery of the egg.

The changes in the contour of the germinal vesicle and its final disappearance can also be followed in the living egg. All these phenomena afford a complete confirmation of the results already obtained from the study of preserved material.

(e) *Fertilization.*

The spermatozoön of *Chætopterus* has a bullet-shaped head and a long vibratile tail. When first teased out into sea-water, it remains motionless for a few minutes, but soon becomes extremely active. Apparently it may penetrate the egg at any point on the surface, though it usually enters nearer the vegetative pole. Polyspermy is rare. The entrance of the spermatozoön initiates profound changes in all parts of the egg. The latent activity of the maturation-amphiaser is revived; the polar globules and the female pronucleus are formed while the sperm is but a very minute and inconspicuous body in a distant portion of the egg.

Other changes are begun in the vicinity of the spermatozoön itself. After it has penetrated a little distance, a diminutive aster with two centrosomes lying close together and surrounded by a minute centrosphere may be seen near it (Fig. 19). These two centrosomes are the sperm-centrosomes, though in *Chætopterus* I am not sure that they are actually carried in by the spermatozoön. However this may be, the sperm-centrosomes separate as the head of the spermatozoön enlarges to form the male pronucleus, and, as they separate, the rays diverging from them become more and more extensive (Figs. 21, 22, 25, 30, 31, 34, 36-38).

Besides moving apart, the centrosomes migrate toward the centre of the egg, the male pronucleus accompanying them, sometimes on one side and sometimes on another, but always near at hand. Their final position is near the centre of the egg, on the side toward the polar globules. The central spindle, which has developed between them, lies at right angles to the egg-axis (Figs. 30, 36-38). After the centrosomes have separated a certain distance, the centrosphere disappears and the rays diverge from the centrosomes themselves. A lightly staining band—the incipient central spindle of the first cleavage-amphiaster—extends from one centrosome to the other (Figs. 36-39).

The rays of the sperm-asters become more and more extensive at the expense of the cytotreticulum until, at the time of the union of the pronuclei, they often extend to the extreme periphery and incorporate nearly all the cytoplasm of the egg. They are not straight but curved, those from either centrosome taking different directions, so that in certain portions of the egg the rays cross one another (Figs. 36-40).

Since the central spindle and two centres of radiation lie on one side of the male pronucleus and in close proximity to it, a conical space on the opposite side of the pronucleus is left free from the rays (Figs. 38-40). In this space and between the rays in other portions of the egg, especially near the periphery, strands of cytoplasm may be seen running in various directions (Figs. 39, 40). The rays themselves are usually branched at the outer ends. The yolk-granules do not approach very near to the centres of radiation, apparently because the rays are too numerous or because the asters repel them. A few granules, however, are found in the conical space opposite the amphiaster and near the pronucleus (Fig. 38).

The sperm-head, or male pronucleus, having grown to its full size, finally takes a slightly eccentric position in that radius of the egg which, if extended, would pass through the polar globules, while near it lie the male centrosomes and the huge amphiaster just described (Figs. 34-40).<sup>1</sup>

<sup>1</sup> In the late stages (Figs. 36-38) there are variations in the relative position of the male pronucleus and amphiaster which show a certain degree of independence



While these phenomena, directly connected with the development of the male pronucleus and amphiaster, have been going on, the first maturation-amphiaster, whose activity was resumed upon the entrance of the spermatozoön, has brought about an apparently independent series of changes in another part of the egg resulting in the formation of the polar globules.

Beginning with the formation of the polar globules, the living egg undergoes a constant series of form changes, these being most pronounced in eggs taken from worms which have been but a short time (one or two days) in the aquarium. While the first polar globule is being formed, the egg, at first spherical, becomes distinctly flattened at the animal pole. This reminds one of the flattening of the adjacent surfaces of the cleavage-blastomeres which occurs shortly after cell-division.

The egg resumes its spherical form, but, after the extrusion of the second polar globule, becomes pear-shaped, the smaller end at the animal pole. It again assumes for a short time the form of a sphere. A protuberance then appears upon the vegetative hemisphere. The successive stages in the development of this protuberance — *yolk-lobe* — are uniformly synchronous with the various phases of the first cleavage-mitosis.<sup>1</sup> The lobe first becomes noticeable during the early metaphase of the spindle, and reaches the height of its development during the telophase. Meanwhile, the first cleavage-furrow cuts the egg into two unequal cells and the lobe remains attached to the larger one, into which it is later resorbed. These phenomena are of especial interest in connection with certain experiments which I have made upon unfertilized eggs.

Soon after the maturation-spindle resumes its activity the nine chromosomes divide and the daughter-chromosomes migrate toward the two poles of the spindle, while the double centrosomes at the inner end of the spindle move apart and a small central spindle is formed between them (Figs. 14-20). The centrosphere fades away and the rays diverge directly from the two centres, as was the case in the evolution of the sperm-

in the behavior of both; but, notwithstanding these variations, the axis of the amphiaster is in every case at right angles to the egg-axis.

<sup>1</sup> The same is true of the yolk-lobe in mollusks; cf. Crampton.



amphiaser at a corresponding stage. The centrosomes at the *outer* end, however, do not move further apart, but are carried into the first polar globule with the nine daughter-chromosomes, and there degenerate (Figs. 19-21).

During the early phases of mitosis the amphiaser presents several interesting features. The yellowish brown fibres of the central spindle may be seen between the chromosomes after they have divided. Between the halves of each chromosome is a distinct white band with no such fibres, but a few minutely beaded, almost black lines (Fig. 17). The spindle does not taper to a point at either end, but is truncated, and at the truncated ends a ring of extremely minute dots, like the centrosomes in color but very much smaller, are brought out in many of the clearest preparations (Figs. 14, 15). These dots are probably nine in number and appear to be the foci of pencils of rays extending to the chromosomes. The latter are clearly seen in transverse sections of the spindle at a little distance from the equator.

In the very late stages of mitosis a delicate *Zwischenkörper* is formed at the junction of the polar globule and egg, but it soon vanishes and has nothing to do with the formation of the second maturation-spindle.

The daughter-chromosomes at the inner end of the spindle, which at first lie in a circle, later take on an elliptical arrangement, while the adjacent centrosomes continue to move apart and eventually lie one at either end of the ellipse (Figs. 22-24). The central spindle, which was formed between the two centrosomes at a very early stage, has now reached considerable size, and we have the incipient amphiaser of the second maturation-spindle.

The centrosomes at the poles of this aster are identical with the two which lie close together at the inner end of the first maturation-spindle (Figs. 14-17), and these are derived by division from one of the centrosomes of the primary asters (Figs. 8-13). The chromosomes soon are drawn into the equator of the spindle, and the latter gradually swings around to a position vertical or nearly so, directly under the first polar globule (Figs. 25-29). Again, a centrosphere develops around

each centrosome, and the rays of the aster—which have never been absent—diverge from it, rather than directly from the centrosome. The centrosome at the inner end of the spindle often, perhaps always, divides, but the daughter-centrosomes are not so large as the corresponding ones in the first spindle, and always remain close together (Figs. 28, 29).

The chromosomes, during the metaphase of the second maturation-amphiasome, are frequently dumb-bell shaped (Figs. 26, 27), and sometimes in four parts, as shown in Fig. 28. The succeeding phases are similar to those of the first maturation-amphiasome described above. The peculiar “wake” left in the midst of the fibres of the central spindle by the receding halves of the chromosomes is evident during the anaphase, and by this means one can ascertain which of the daughter-chromosomes were together (Fig. 29). It is found in some instances that one of the chromosomes is drawn much nearer the pole of the spindle than its counterpart.<sup>1</sup>

The second polar globule is formed directly under the first, which is thus pushed away (Figs. 31–33), and it contains the centrosomes and the nine daughter-chromosomes (Fig. 31).

At about the 32-cell stage, both polar globules are ingested by the apical cells.<sup>2</sup> The *Zwischenkörper*, which is developed during this mitosis, persists for a considerable time, as may be seen by comparing Figs. 31, 32, 35, 37, and 39.

As the spindle vanishes the chromosomes which were at the inner end elongate and bend so as to become V-shaped (Figs. 31–33). They then group themselves in a hollow hemisphere whose concave side is directed toward the centre of the egg so as partially to obscure the centrosome and its vaguely defined centrosphere, though at this period the radiations from the aster in question are extensive and very distinct, many of them crossing those of the male aster. The chromosomes ultimately surround the centrosome so that the astral rays diverge from their very midst (Figs. 33–35).

Gradually the chromosomes become vesiculated and, as the nine vesicles begin to migrate toward the male pronucleus, they

<sup>1</sup> In this figure the lithographer has slightly displaced the outer centrosomes and the chromosome nearest the pole.

<sup>2</sup> Cf. Mead, '97b.

continue to grow and press against one another, still including the female centrosome, whose position is indicated by the point of convergence of the rays (Figs. 35-38). The latter gradually become fewer and less distinct and finally vanish altogether. I believe that the rays of the female aster, which were so strongly developed in the earlier stages of the reconstitution of the pronucleus, become resolved into a cytoplasmic network, which in part may be incorporated into the system of rays belonging to the male amphiaster. I have seen during this period of disintegration a number of extremely minute asters (secondary or tertiary mechanical centres) between the periphery of the egg and the female pronucleus.

The vesicles completely coalesce to form a female pronucleus similar in size and general appearance to the male pronucleus with which it later comes into close apposition (Fig. 39).

These phenomena suggest the interpretation that the chromosomes at the inner pole of the second maturation-spindle are not only drawn toward the egg-centre, but that the latter continues for some time to be the centre of attraction and thus groups the chromosomic vesicles about itself and holds them together. The disappearance of the female aster is simultaneous with the coalescence of the vesicles. After their fusion there is no further need of this attractive influence and the aster disappears. Apparently, also, the male centres exert an attractive influence upon the group of vesicles as a whole, though it is not ordinarily strong enough to dissociate the group by drawing the nearest vesicles to the centre in advance of the others. In one case, however, this phenomenon seems actually to take place (Fig. 36).

But, whether the grouping of the vesicles is a function of the female centrosome or not, it would seem utterly preposterous to presume that this waning structure suddenly emerges from the midst of the fusing vesicles, divides, and unites with the male centrosomes.

Before the two pronuclei meet, the vesicles of the female are completely united and resemble the male pronucleus in all respects, though they always lie on the side toward the polar globules. The two pronuclei come together and flatten against

each other between the poles of the male amphiaster, forming a spherical nucleus — the first cleavage-nucleus (Figs. 39, 40).<sup>1</sup>

The male asters reach the height of their development as the pronuclei come together, and the two centres, connected by a spindle, are already widely separated. Each centre contains a clearly defined and easily demonstrable centrosome, which is soon surrounded by an incipient centrosphere, from which the protoplasmic rays extend throughout the whole egg (Figs. 39, 40). The pronuclei elongate, while the poles of the spindle continue to move further apart, and later the nuclear membrane gradually disappears. An actual fusion of the pronuclei does not take place (Figs. 40, 41), and, even after the membrane has vanished, the chromosomes derived from the egg and from the spermatozoön respectively are often seen to be in separate groups (Fig. 43). As the cleavage-nucleus begins to elongate, the rays of the amphiaster become less extensive, breaking up into a network in the outer portion of the cytoplasm, and when the nuclear membrane disappears, centrospheres become more strongly developed around the centrosomes. The chromosomes arrange themselves in an equatorial plate, and the metaphase of the first cleavage-amphiaster is established (Figs. 43, 44).

The centrosomes at the poles of the spindle by this time have each divided in anticipation of the next mitosis, and the centrospheres have increased in size. Several nucleoli lie scattered among the chromosomes in the equatorial plate (Fig. 45). Like the chromosomes they stain with hæmatoxylin, but are easily distinguished by their irregular shape and arrangement (Fig. 46).

The chromosomes split longitudinally according to the heterotypical method (Figs. 44, 45), and the daughter-chromosomes recede toward the opposite poles (Fig. 46). In the cross-section of the spindle I have often counted the chromosomes, and found them always to be eighteen in number (Fig. 47), as would be expected, since there were nine in the maturation-spindle (Fig. 18). When the daughter-chromosomes have separated to some distance, the nucleoli are seen in their original position midway between the poles of the spindle (Figs. 46,

<sup>1</sup> The signs ♂ and ♀ in Figs. 40, 41, and 43 should be transposed.



46<sup>a</sup>), where they remain during the succeeding stages of mitosis (Figs. 48-52).

The centrospheres reach their greatest development toward the end of the anaphase, though the radiations at this time are not so extensive (Figs. 46<sup>a</sup>-48). The centrosomes, which divided very early, continually move apart within the centrosphere without in the least altering the regular contour of the latter. In the early stages a line joining the two centrosomes would nearly coincide with the spindle-axis, but as they move apart they also swing around through an angle of about 90° (Figs. 44-48). The distance between the centrosomes within the centrosphere is in definite and constant relation to the successive phases of mitosis, and one can predict the position of the chromosomes from the examination of the centrosomes, and *vice versa*. The centrosphere is smaller in the end of the spindle which belongs to the smaller cell (Figs. 46, 46<sup>a</sup>, 48). The dark brown centrosomes are especially distinct during these stages in contrast to the light yellow centrosphere. In some preparations a minute halo surrounds each centrosome (Fig. 46), and in the later stages of the anaphase, before the centrosome disappears, a band of fibres, the incipient central spindle of the next mitosis, is demonstrable between them (Figs. 46<sup>a</sup>, 47).

When the rod-like chromosomes have reached a position near the centrosphere they gradually become vesiculated. Each chromosome at first appears as a double row of granules, separated by a longitudinal cleft (Fig. 48), but later the regular arrangement of the granules is lost, and each chromosome continues to swell up and forms an oval vesicle (Fig. 49) resembling a miniature nucleus. I have several times counted the vesicles, and always found them to be eighteen. They arrange themselves in the form of a disc, and for some time the fibres of the centrosphere may be seen between them (Fig. 49). As they grow still larger they coalesce, not into a single mass, but into several irregular masses which themselves fuse to form a spherical nucleus (Figs. 50, 51).

The cytoplasmic portion of the egg becomes constricted during the later phases of the reconstitution of the nuclei, and



in the line between the two new cells there appears a series of black granules, the foci of pencils of connecting fibres extending in both directions to the nuclei (Fig. 51). These bodies later become aggregated at one place and form a large brown *Zwischenkörper* (staining like a centrosome), from which the rays diverge. In side view the *Zwischenkörper* may always be seen to lie below a line connecting the centres of the nuclei; *i.e.*, nearer the vegetative pole. At this stage the nucleolar fragments of the original cleavage-nucleus lie in the larger of the two cells (Figs. 51, 52). Here they remain for a while, gradually becoming smaller and less distinct, and vanish entirely before the karyokinetic figures are formed in the two blastomeres.

The centrosomes, which divided at an early stage and separated as the karyokinesis progressed, continue to move apart during the reconstitution of the nuclei, and a spindle develops between each pair. While the chromosomes are being transformed into vesicles the centrosphere at either end of the spindle disappears (Fig. 49), and the rays which now diverge from the centrosomes increase rapidly in length and thickness, and reach their maximum development in a late stage of the reconstitution of the nucleus, as is represented in Figs. 51 and 52. They extend to the periphery of the egg and are easily distinguishable between the closely packed yolk-granules at the lower pole. They can be traced even through the substance of the yolk-lobe which develops on the lower hemisphere when the first cleavage-spindle is in an early stage (metaphase) and eventually becomes a part of the larger of the two blastomeres (see Fig. 50).<sup>1</sup> The rays diminish in extent as the new nucleus assumes its definitive spherical contour; the centrosomes, meanwhile, take their respective positions, nearly 180° apart, near the surface of the nucleus (Fig. 53).

The reconstitution of the nuclei and the accompanying phenomena proceed simultaneously in the two blastomeres, and each is now in a stage of karyokinesis exactly comparable to that of the original oöperm after the union of the pronuclei.

<sup>1</sup> In a previous account of this phenomenon I said that the yolk-lobe was separated from the blastomere. This was a mistake, which I corrected in a later paper.

One cycle of karyokinesis has been completed, and the next cycle which is concluded with the formation of the four blastomeres is essentially similar. Around each centrosome there develops a centrosphere (Fig. 53). The nuclear membrane, beginning at the portion nearest the centrosome, breaks down (Fig. 54), the chromosomes group themselves in the equatorial plate (Figs. 55, 56), and the typical anaphase is established. During the succeeding stages, the chromosomes in each blastomere divide longitudinally and migrate toward the poles of the spindle, the nucleoli drop out into the cytoplasm, and the centrosomes divide and move apart within the growing centrosphere (Figs. 57, 58).

I have followed the karyokinetic processes to the formation of sixteen cells. All the phenomena are essentially similar to those in the preceding cycles of division. The thirty-two centrosomes of the 16-cell stage arise by the successive divisions of the original sperm-centrosome, while the centrospheres, on the other hand, appear and vanish in each mitosis.

### III. SUMMARY.

During the growing-period of the oöcytes a deeply-staining paranucleus is developed which contains a reticulum continuous with that of the surrounding cytoplasm; but, before the oöcyte has attained to its full size, this structure becomes entirely resorbed into the general cytoreticulum.

Ripe eggs may be carried in the body-cavity of the worm for several days before they are laid. During this time neither centrosome nor aster can be distinguished, though the reticulum is unusually distinct. In a few minutes after the eggs have been deposited in sea-water, however, a large number of asters are developed by rearrangement of the cytoplasmic network.

Two of these asters (primary asters) continue to develop and finally lie at the poles of the first maturation-spindle, while the others gradually vanish. Distinct centrosomes are not demonstrable except in the primary asters. If they are present in the multiple asters, it is extremely difficult to reconcile their presence with the theory that the centrosome is a permanent

cell-organ; for the theory would require, in this instance, that no less than seventy-five centrosomes should arise by the division of the two centrosomes of the cell of the preceding generation, and that these centrosomes should be distributed throughout the larger portion of the egg-cytoplasm. If they are not present in these asters, the centrosomes are only an occasional and not a constant or an essential feature of the aster.

The centrosomes of the two primary asters evidently arise *de novo* out of the cytoplasm, and are typical in every respect; they lie in the midst of the astrospheres, grow, divide, and persist in the next cell-generation.

Normally the maturation of the unfertilized egg proceeds no further than the metaphase of the first maturation-spindle, but upon the entrance of the spermatozoön the karyokinetic activity is immediately resumed and the maturation is completed. I do not know whether the spermatozoön actually brings the sperm-centrosomes into the egg or not; at any rate, they are demonstrable in the midst of a minute aster which lies close to the sperm-head soon after the latter enters. From this time the development of the sperm-aster into the cleavage-amphiaster, and the mitotic divisions, resulting in the formation of the polar globules, proceed simultaneously in different parts of the egg, and appear to be independent phenomena.

The primary centrosomes lie at the poles of the first maturation-spindle, and the daughter-centrosomes, arising by the division of one of these, move apart and form the poles of the second maturation-spindle. During the reconstitution of the egg-nucleus and its approach to the sperm-nucleus, the egg-centrosome remains in the midst of the fusing vesicles, its position being indicated by the point of convergence of the rays of its waning aster. Not only the fact of its disappearance, but the fact that, when last seen, the centrosome is in the midst of the group of vesicles, renders it in the highest degree improbable that the egg-centrosome takes part in the formation of the cleavage-amphiaster. Moreover, the sperm-centrosomes may always be seen at the poles of the incipient cleavage-amphiaster, and they become more and more conspicuous up to the time of

the fusion of the pronuclei. During the metaphase of the cleavage-amphiaster the sperm-centrosomes divide, and the daughter-centrosomes at either pole move apart (an incipient central spindle developing between them) and form the poles of the cleavage-spindles of the two blastomeres. This process is repeated in each subsequent mitosis, and the centrosome can be demonstrated, lying in the midst of an aster, at every phase of mitosis, even including the so-called "resting stage." It follows, therefore, that the centrosomes of the cleavage-cells are derived directly from the sperm-centrosomes, — a fact irreconcilable with Fol's theory of the "quadrille."

The behavior of the sperm-centrosomes is in harmony with Boveri's theory of fertilization, but is not necessarily a confirmation of it; for the karyokinetic activities which are revived upon the entrance of the sperm are those leading to the formation of the polar globules. The machinery for these mitotic divisions is already organized, and it is quite as likely that the stimulus which starts it going emanates from the sperm-nucleus as that it emanates from the sperm-centrosomes.

There are nine chromosomes in each maturation-amphiaster and eighteen in the cleavage-spindle. In the metaphase of each cleavage-amphiaster the numerous nucleoli lie scattered among the chromosomes and remain at the equator of the spindle until they completely degenerate. As mitosis progresses, the chromosomes split longitudinally and the halves move toward opposite poles of the spindle, where they form new nuclei.

During the telophase of each mitosis a distinct *Zwischenkörper* is present, which gradually fades away as the reconstituted nuclei approach the "resting stage."

It is a pleasure to acknowledge the many courtesies extended to me by Dr. Whitman and other officers of the Marine Biological Laboratory, and to express my appreciation of the kind assistance of my friend Dr. W. M. Wheeler.

BROWN UNIVERSITY,  
September 26, 1897.



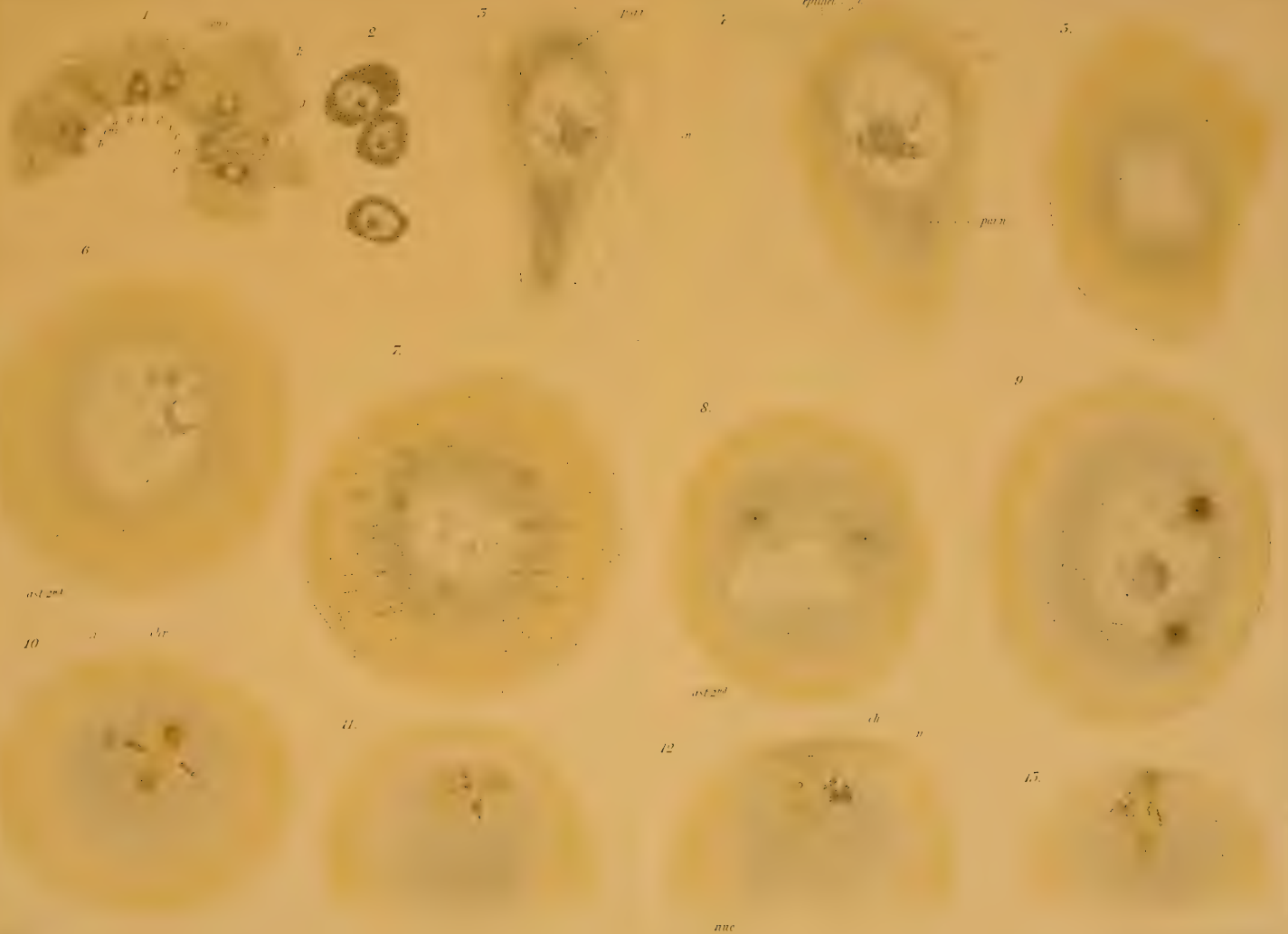
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## EXPLANATION OF PLATE XVI.

Unfertilized ovarian eggs. Except where otherwise stated, the figures are drawn from single sections.

FIG. 1. Transverse section of ovarian tubule, showing relative size, position, and color of ova in different stages of development, *a-k*. Paranucleus in *c-g*. Beginning of formation of asters in *i* and *j*, which are well developed in *k*; *ep.o.*, outer epithelium of ovary; *ep.i.* inner epithelium.

FIG. 2. Small ovarian eggs, *a* and *b* of Fig. 1; same scale of magnification as rest of figures.

FIG. 3. Compare with stage *f* of Fig. 1; *par.n.*, paranucleus; *n.*, nucleolus.

FIG. 4. Last traces of the paranucleus.

FIG. 5. Slightly later stage than Fig. 4. Paranucleus has become completely resolved into the cytotreticulum; compare *h*, Fig. 1.

FIG. 6. The cytoplasmic network beginning to form small asters. On the right the pellicle is torn away from the rest of the reticulum. Compare *i* and *j*, Fig. 1.

FIG. 7. Numerous secondary asters at the height of their development. Compare *k*, Fig. 1.

FIG. 8. The two predominant primary asters with centrosomes. Beginning of the inpushing of nuclear membrane. Several small secondary asters' still visible.

FIG. 9. Egg with only the two primary asters; each with a clear centrosome, (dark brown). Distinct inpushing of nuclear membrane. Eggs at this stage are no longer attached to the ovary.

FIG. 10. First maturation-spindle lying on the surface of the nucleus; nuclear membrane dissolved. Chromosomes and nucleolus outside the spindle; centrosomes in each aster doubled and smaller than in Fig. 9.

FIG. 11. Side view, similar to Fig. 10. Remains of germinal vesicle in middle of egg. The chromosomes, which appear in the drawing to be in the midst of the spindle, are seen by focusing to lie above the central spindle. In the right-hand aster only one centrosome is visible; the other is probably directly below it.

FIG. 12. Later; the spindle has swung around to a vertical position; *n.*, nucleolus; *ch.*, chromosomes. Both centrosomes are visible in the inner aster, one lying almost underneath the other, and therefore farther apart than they appear in the figure. The last remnants of the nucleus *nuc*.

FIG. 13. Chromosomes of more definite shape and more evenly distributed around the equator of the spindle. Spindle more elongated and nearer the periphery of the egg. Remains of nucleolus on the left.

## EXPLANATION OF PLATE XVII.

With the possible exception of Figs. 14 to 16, all are fertilized.

FIG. 14. First maturation-spindle after the disappearance of nucleoli. Double centrosome in each end. Circle of dots around end of spindle. (Two sections combined.)

FIG. 15. Similar to Fig. 14; centrosomes of inner aster further apart; circle of dots more deeply stained.

FIG. 16. Circle of dots not brought out, though the centrosomes are clear. There are probably two centrosomes at the outer end of the spindle, though only one shows in this section.

FIG. 17. About ten minutes after fertilization. Daughter-chromosomes moving toward the pole of the spindle. The two centrosomes at the inner end of the spindle moving apart, showing a faint whitish band (not clear in the lithograph) between them.

FIG. 18. Transverse section through the equatorial plate, showing the nine chromosomes.

FIG. 19. First maturation-spindle in anaphase. Polar globule beginning to protrude. Young sperm-nucleus, ♀ with its single (?) centrosome.

FIG. 20. The first polar globule. One of the outer centrosomes of the first maturation-spindle degenerating, but still visible. The two inner centrosomes widely separated, with an incipient spindle between them. A mere suggestion of a *Zwischenkörper* in the large spindle, *z*. The sperm-nucleus as in Fig. 19. This figure shows the distinction in color between the blue-black chromosomes and the brown-black centrosomes which is always present in the preparations themselves. Sperm-nucleus from adjacent section.

FIG. 21. Oblique view; first polar globule, *Zwischenkörper*, male pronucleus with aster and two centrosomes. Remains of centrosome in the polar globule. Combined from several sections. The full number (9) of chromosomes in the egg.

FIG. 22. Oblique section; after the evanescence of the first maturation-spindle. Some of the chromosomes at inner end of the first spindle; compare Fig. 20. The two centrosomes more widely separated, between them the incipient central spindle; *p.g.*, polar globule in tangential section. Two sperm-centrosomes.

FIG. 23. Incipient second maturation-spindle; slightly later than Fig. 22, not all the chromosomes in this section.

FIG. 24. Two adjacent sections *a* and *b*; *p.g.*, first polar globule with chromosomes fusing together; second maturation-spindle far advanced.

FIG. 25. Second maturation-spindle beginning to swing around into vertical position. Sperm-nucleus with aster and two centrosomes.

FIG. 26. The second maturation-spindle in its final position. Aster of sperm-nucleus with extensive rays.

FIG. 27. Second maturation-spindle a little later.

FIG. 28. Same, with quadruple chromosomes. Circle of dots around end of spindle; compare Figs. 3, 14, and 15.

FIG. 29. Second maturation-spindle; compare Fig. 17. One chromosome near outer pole of the spindle. All the daughter-chromosomes can be seen in this section by focusing.

FIG. 30. A little later stage, paired sperm-centrosomes; whitish band between the two centrosomes. Centrosomes of maturation-spindle indistinct.

FIG. 31. Telophase of second maturation-spindle. Degenerating centrosomes in the second polar globule, *p.g.*<sup>2</sup>; *Zwischenkörper*, *z.*; sperm-nucleus,  $\mathfrak{F}$ , with two centrosomes which are not so widely separated as in Fig. 30, though here the maturation processes are farther advanced (all one section  $3\mu$ ).

FIG. 32. About the same stage; shows the more usual conditions in that the second polar globule, *p.g.*<sup>2</sup> pushes the first away from egg. Egg membrane, *m.*; *z.* *Zwischenkörper* or middle-plate.

FIG. 33. Later than Fig. 32. The chromosomes at inner end of second maturation-spindle in the form of loops and partly surrounding the centrosome and aster. It is possible that there are two centrosomes. The location of the centre of the aster is indicated by the point of convergence of the rays, which at this time are very prominent.













## EXPLANATION OF PLATE XVIII.

FIG. 34. Two sections combined as indicated by the arcs of circles. First polar globule lost, second polar globule bent over. Male pronucleus and aster with two centrosomes in the centre of the egg.

FIG. 35. Two polar globules,  $pg.^1$  and  $pg.^2$ ; *Zwischenkörper*, *z.* Cluster of vesicles,  $\varphi$ , formed from chromosomes left in the egg after the second maturation-division. In the midst of the vesicles lies the centrosome towards which the rays converge. In the midst of the egg the male pronucleus and aster with one of the two centrosomes in sight (the other in the next section). The rays of both sperm and egg-asters break up near the periphery into the network.

FIG. 36. The two male asters with centrosomes and long rays are in definitive position; sperm-nucleus,  $\delta$ , in rather unusual position, *i.e.*, between asters and female pronucleus, the vesicles of which are larger than in preceding figure. Anomalous case of one vesicle reaching the male pronucleus before the others. Aster with rays (two sections combined).

FIG. 37. Two polar globules, 1 and 2; *Zwischenkörper*, *z.* Vesicles of female pronucleus very large and close together. Male pronucleus in rather unusual position at side of asters. Spindle between the latter. Rays of asters extend to the periphery and converge to the centrosomes themselves, as in preceding figure; no centrosphere.

FIG. 38. Later than Fig. 37. Vesicles of female pronucleus uniting, a few faint rays converging to their midst. Male pronucleus and asters in usual position. (Two sections combined.)

FIG. 39. Two polar globules, 1 and 2; *Zwischenkörper*, *z.* Male and female pronuclei about to unite. Two male centrosomes and asters. Female aster has entirely disappeared.

FIG. 40. Male and female pronuclei have united. The male asters on either side. Centrosomes are clear and centrospheres are beginning to form about them. The rays diverging from the latter are commencing to break up into a reticulum near the periphery. Round nucleoli in the pronuclei. Signs  $\delta$  and  $\varphi$  transposed.

FIG. 41. First cleavage-nucleus elongated; its membrane commencing to break down at the ends. The two sperm-asters with centrosomes and centrospheres; rays comparatively short. Signs  $\delta$  and  $\varphi$  transposed.

FIG. 42. Somewhat later stage. Mere traces of nuclear membrane now visible. Chromatin, nucleolus, *n.*, in middle, centrosomes and centrosphere larger than in preceding figure.

FIG. 43. Cleavage-spindle in metaphase. Chromatin in two clusters, and nucleoli scattered among the chromatin loops. Centrosome in right aster elongated in preparation for a division. Signs  $\delta$  and  $\varphi$  transposed.

FIG. 44. Later stage. Centrospheres more highly developed; centrosomes dividing in anticipation of next cleavage.

FIG. 45. Daughter-chromosomes divided and beginning to migrate toward the two poles of the spindle, leaving nucleoli in the middle. Daughter-chromosomes moving apart.







34.  $pp^2$

35.  $z$   $pp^1$   
 $pq^2$

36.

37.

38.

39.

40.

41.

42.

43.

44.

45.

poll





## EXPLANATION OF PLATE XIX.

FIG. 46. First cleavage-spindle in the metaphase. Chromosomes moving towards the poles, with nucleoli left in the middle between them. Centrospheres at the height of their development.

FIG. 46a. Late anaphase. The centrosphere at left shows the destructive action of corrosive-acetic acid.

FIG. 47. Two adjacent sections combined. One shows the centrosphere and two brown-black centrosomes with central spindle between them; the other, the eighteen chromosomes, black, belonging to the larger aster. The smaller aster showed the same features.

FIG. 48. First cleavage-spindle in the beginning of telophase; chromosomes commencing to swell up into vesicles. Nucleoli, *n.*, centrospheres, and centrosomes as in the two previous figures.

FIG. 49. Spindle in the telophase; chromosomes vesiculated; fibres in the middle of spindle very distinctly varicose and bent; nucleoli in the midst of spindle. Centrospheres completely resolved so that the rays diverge from the centrosomes directly and are again very extensive, many of them reaching the periphery of the egg. Yolk-lobe, *y.l.*

FIG. 50. Transverse section through the larger cell a little later than the preceding figure. Yolk-lobe, *y.l.* Two adjacent sections combined as in Fig. 47. The two centrosomes with central spindle between them. Eighteen chromatic vesicles.

FIG. 51. Reconstitution of the nuclei. Only one of the two centrosomes in either cell is visible in this section. Middle-plate, *z.*, in many pieces from each of which diverging fibres extend to the nuclei. Nucleoli or first cleavage-nucleus remaining in larger cell, *n.*

FIG. 52. Three consecutive sections, 1, 2, 3, combined: *z.*, *Zwischenkörper*; *n.*, nucleoli of first cleavage-nucleus; *n.1*, nucleoli in new nucleus. Both centrosomes visible in either cell.

FIG. 53. Smaller of the two cells with resting nucleus. Two asters on opposite sides of nucleus; centrosomes, centrospheres, and comparatively short rays.

FIG. 54. Transverse section of one of the two cells. Nuclear membrane breaking down. Cytoplasmic network; nuclear network; chromosomes, *ch.*; nucleolus, *n.*; two centrosomes and central spindle.

FIG. 55. Transverse section of one of the two cells. Prophase of the second cleavage-spindle; chromosomes, *ch.*; nucleolus *n.*

FIG. 56. Beginning of the metaphase, 2-cell stage. Chromosomes in both centrospheres dividing.

FIG. 57. Transverse section of the smaller of the two cells. Chromosomes undergoing heterotype splitting; centrosomes distinctly separate.

FIG. 58. Transverse section of the larger cell of the same egg as Fig. 57. Spindles slightly further advanced. Centrosomes at a considerable distance apart in both asters.



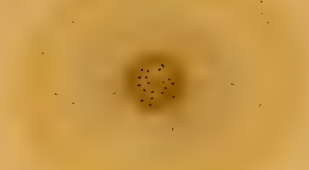




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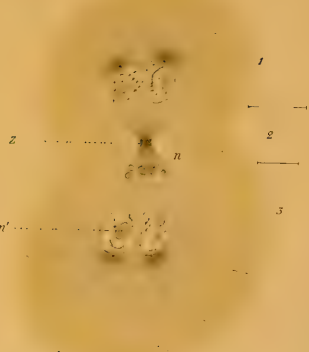
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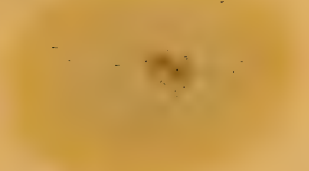
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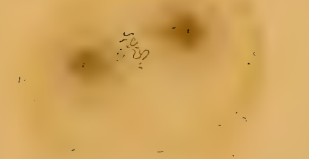


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# THE EMBRYOLOGY AND OÖGENESIS OF ANURIDA MARITIMA (GUÉR.).

AGNES MARY CLAYPOLE.

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THE form chosen for this investigation is the small Collembolan, *Anurida maritima*; it is a form of very wide geographical distribution. It has been reported from many different places on the eastern and western shores of the Atlantic Ocean, and is probably present at many others. It received its specific name in Europe from the French naturalist Guérin in 1829-38. It has also been called *Achorutes maritimus* by Grube and *Lipura maritima* by Lubbock. According to the latest publications, *Anurida* belongs to the group Lipuridae, which, together with the Poduridae and Smythuridae, form the suborder Collembola. Specimens were sent to Dr. H. T. Fernald, who confirmed their identification as *Anurida maritima*. The only contribution on the subject already published is one by Ryder in the *American Naturalist* for 1886, where



an adult, recently hatched young, and several stages of embryos are figured and described. All of Ryder's studies were made on whole specimens, and none of the early stages were figured.

The material was collected mostly during the summer of 1895 at Woods Holl, Mass., in connection with work done at the Marine Biological Laboratory. Both animals and eggs are found under stones, on sandy beaches, at about the level of half-tide, so that they are completely submerged for about half the time. They would thus be submerged to a maximum depth of about eighteen inches of water, the average tide being three feet in height.

Different methods of killing and preserving eggs and adults were used. The most satisfactory for the eggs proved to be the simple process of killing in hot water and hardening first in 70% and then passing to 95% alcohol. Owing to the thick membranes surrounding the ova, other processes failed for lack of penetration and resulted in shrinkage and distortion. Adults and young ones were killed in hot water and hardened in the same way as the ova; those killed and hardened in warm and hot corrosive-acetic and picro-acetic acids and kept afterwards in 95% alcohol gave very good results. Owing to the difficulty experienced in wetting them, any method involving an early application of alcohol was easier than using water solutions entirely. Hot solutions were found better than warm, as they prevented contraction, causing the animals to die stretched out straight.

The material was almost all cut in hard paraffine and stained on the slide variously with borax carmine, Erlich's haematoxylin, lithium carmine and Lyon's blue, iron haematoxylin and orange G. Double staining was chiefly used and a few tests made with Biondi-Erlich. Sections were cleared in xylol and mounted as usual. In thickness they varied from five to fifteen micra.

#### *Structure of Adult Ovary.*

In specimens in which the eggs are nearly mature the structure of the ovaries is much obscured, as the abdomen appears to consist of a mass of eggs closely packed together and hence

irregular in shape. These eggs are not spherical nor even oval, but angular and compressed so as to entirely fill the abdominal and even part of the thoracic cavities. The number of eggs in a single individual varies from twelve to twenty; among the large mature eggs, which appear as masses of yolk, there are numerous small, immature ova, and in certain places masses of germinal epithelium.

Taking a stage where the eggs are less mature and yolk formation less advanced, the structure of the ovaries can be made out definitely. The organs are composed of a pair of simple tubes placed one on each side of the alimentary canal, as described by Fernald ('90). These are prolonged anteriorly into two long filaments, which reach into the second thoracic segment and finally coalesce with the fat body on each side at about the level of the middle of the alimentary canal in the vertical plane. Posteriorly the tubes retain their paired condition to the end of the fourth abdominal segment; at this point the tubes unite and form a single receptacle that may be called the uterus. This shortly passes into the oviduct, which is a median and unpaired opening at the hinder end of the fifth segment, and then directly to the ventral surface of the animal. Below this oviduct, as described by Fernald ('90), is a small sac opening to the outside by a common opening with the oviduct, which may be a receptaculum seminis; but careful search in many stages failed to reveal the presence of any spermatozoa.

Fig. 1, Pl. XX, is a longitudinal section through an ovary in which the eggs are just beginning to accumulate yolk; it shows the cephalic elongation, egg masses, and germinal epithelium. Nothing of the unpaired uterus and duct can be seen as the section passed to one side of these parts.

Four different structures are clearly shown. First, the cephalic elongation (Fig. 1, *c.el.*), which runs into a fine point and is attached laterally to the fat body lining the thorax. The structure of the thread is shown in Figs. 1, 5, and 6. It is a fine membrane in which nuclei are irregularly placed. These nuclei have no germinal characters; they are not to be distinguished from the nuclei of the wall of the ovary. This

elongation ceases in the middle or near the beginning of the first abdominal segment, and the ovary proper begins. This is in structure a simple tube, as shown in Fig. 1, in which are found many cells grouped round masses of protoplasm that differentiate clearly by staining in Lyon's blue. These cells are large and remarkably rich in chromatin of a peculiar arrangement. No nuclei are visible in the protoplasmic masses in the stages shown in Figs. 1 and 10, but in an earlier stage (Fig. 4), when yolk formation has not yet begun at all, a small, much-shrunk nucleus is distinctly visible. Caudad of these cell clusters lying in the third to the fourth abdominal segments is a large group of cells crowded together with indistinct outlines (Fig. 1, *g.e.*). This is easily recognizable as the germinal epithelium, and lies distinctly separated from the rest of the cells in the ovary. There is no definite arrangement of cells in this mass excepting a tendency to run in rows, which may lie in any direction, longitudinally, transversely, or dorso-ventrally. The germinal mass is evidently non-metameric; there is no division of it into parts, and the whole is included within the third and fourth body segments. Numerous karyokinetic figures in the mass show that active proliferation is in progress. It is among the cells of some of these outer strings that the first differentiation is seen. Typically, the germinal cells are small and spherical; the cell bodies are very small and the nuclei large with the chromosomes regularly arranged on the periphery. Fig. 9 shows the first appearance of differentiation; in a line of cells among the typical round-nucleated cells appears one with a smaller, more oval nucleus containing smaller chromosomes which are still on the periphery. The development of a string of cells such as is shown in Fig. 9 into an egg mass can be followed in Figs. 2, 8, and 9. When the stage represented in Fig. 8 is reached the group of cells has passed to the outside edge of the germinal epithelial mass and is ready to fall freely into the ovary.

Returning for a more detailed consideration of the cell clusters, it is evident, as shown in Figs. 1, 2, 4, and 8, that there are many cells associated with that mass of protoplasm which contains, in early stages, the small, shrunken egg nucleus

(Fig. 4). This associated mass ultimately becomes the egg, the protoplasm containing the nucleus forming the definitive ovum and the surrounding cells bearing the relation of nutritive cells. Of the latter there are usually from five to eight grouped round one ovum; in the early stage they are not distinguishable from the general mass, but at a certain time an increase in size and also in the proportionate amount of chromatin is marked. This, together with the simultaneous decrease in size and change in shape and in amount of chromatin of the ovum nucleus, serves to emphasize the suddenness of differentiation. Fig. 10 shows the characteristic position of the ovum surrounded by a semicircle of nutritive cells. The change in the appearance of the chromatin can readily be seen by comparing Figs. 2, 9, and 10. In Figs. 2 and 9, the youngest stages, the chromosomes are rather small, irregular masses on the periphery, usually eight in number, with a more centrally placed nucleolus. In the later stage (Fig. 10) the chromosomes have assumed a stellate structure of a most pronounced type; they are usually eight in number with a more central and non-stellate nucleolus. In the same figure there is an additional change. There appears in the cell body close to the nucleus a mass of material clearly of a distinct chemical nature. It forms a cap, as it were, on one side of the nucleus, usually the side next to the ovum; by its accumulation it pushes the nucleus out of its hitherto central into an eccentric position. The presence of this material in the nutritive cells indicates a young stage in the ovum; yolk has not yet appeared in any large quantities.

One more point must be noticed in this connection. There is always associated with the nutritive cells, usually one on each end of the string, a cell of distinctly different character. This is shown at *a.o.* in Figs. 10 and 11. It entirely lacks the characters of the nutritive cell. It is smaller, has an extremely small cell body and large nucleus with eight minute chromosomes, which are at first arranged on the periphery, as is the case with the nutritive cell. There are one or two large nucleoli easily distinguishable from the eight chromosomes. Later the chromosomes move inward and arrange themselves in groups of four (Fig. 13). These cells have without doubt



some of the characteristics of the true ovum, and are ova that have passed in the course of development up to a certain point. The nuclei must then have undergone a degenerative hypertrophy, as they are much larger than those of the developing ovum of the corresponding stage. The cell body grows smaller and takes the Lyon's blue stain strongly on double staining after lithium carmine. The ultimate fate of these cells is the same as that of the nutritive cells, — degeneration and absorption.

The last stage in which the nucleus of the ovum is recognizable is shown in Fig. 4. The egg now consists of an irregular vesicular mass of protoplasm which forms the cell body in which a very small nucleus or germinal vesicle is eccentrically placed. This nucleus is irregular in outline with very small, peripherally arranged chromosomes, the number of which it was impossible to determine exactly; by inference it would be eight. The germinal vesicle has every appearance of losing its nuclear membrane; if this is the case, its apparent disappearance after this stage is attributable to the difficulty of distinguishing the separated minute chromosomes. In confirmation of this point a few observations were made on another apterygote, a thysanuran (*Tomoceras* sp.?), in which eggs were found in a similar stage as well as some a little more advanced. The cells are much larger in these animals and the ova are distinctly large masses of vesicular protoplasm with a small group of eight chromosomes eccentrically placed, not surrounded by any nuclear membrane (Fig. 14, *g.v.*).

In the next stage in Anurida, as shown in Fig. 10, the nucleus is no longer visible, the protoplasm has increased in quantity, and has also become more vesicular. In some of the spaces of the network yolk has already begun to appear, as is shown by the deep staining of parts in Lyon's blue. The nutritive cells have grown in size and the stellate structure of the chromatin is clearly defined. That these stellate masses are the chromosomes is shown by the use of the Biondi-Erich stain. In this the stellate masses appear dark green, almost black at the central, more dense spot, and lighter green in the radiating strands; the non-stellate masses take the bright red



of nucleoli. These masses vary in number from one to two. The cell bodies of the nutritive cells have increased greatly in size, and at this stage show the differentiation into two kinds of material most clearly. By the use of lithium carmine and Lyon's blue the contrast is rendered most vivid. The chromosomes and nucleoli stain a brilliant red in the carmine, differentiating the nucleolus as a peculiarly refractive body. The cell body protoplasm stains a rather purplish red, being affected to a slight degree by both stains. The cap of different material comes out a brilliant blue, as also does the yolk present in the protoplasmic meshes of the ovum. Although no membrane can be found delimiting this blue material, its edge is as clear cut as if such a condition existed. From the constantly circular appearance of the nuclei of the nutritive cells when cut in all planes it is evident that they are spherical, and hence the blue material must cover the nuclei as a cap on the side facing the egg, thus causing the nucleus to take an eccentric position.

While these changes are taking place in the ova and nutritive cells the wall of the ovary begins to grow round each of the cell masses, and finally the ovum and the cells that contribute to its formation are inclosed together in a more or less completely separate sac (Fig. 4, *f*). During the period of yolk formation the follicle is gradually stretched more and more, and hence becomes thinner and thinner. There is no evidence that the follicular cells divide either kinetically or akinetically, as has been described by Wheeler ('39) in *Doryphora* and *Blatta*. This thinning process is continued until, as shown in Figs. 11 and 12, the follicle is hardly distinguishable. At no time is it a prominent feature of the ovary, and it becomes more and more insignificant from the beginning of yolk formation. Its slight development is a most potent argument against its taking any active part in yolk formation. Commonly the follicular cells either completely or in part supply the elements of this process. In many cases, as shown by Stuhlmann ('36) and others, a highly developed follicle is present; also an ovarian wall and nutritive cells. As a rule, however, the degrees of the development of the follicle and the nutritive cells stand in inverse ratios, the work of aiding the egg in

yolk secretion being performed by either one or the other. In the case of *Anurida* the inference is that the nutritive cells take an active part in the yolk formation. Their great growth during the early stages of the egg, the presence of differentiated material in them, its disappearance, and, following this, the consequent appearance of yolk in the egg, the continued vigor of these cells until the full size of the egg is attained, and then their gradual absorption, are all facts pointing to the functional activity of the nutritive cells and their direct relation to yolk formation.

Fig. 11 shows the nutritive cells at their stage of greatest development. The increase in the size of the eggs and the consequent crowding in the body cavity have together given the nutritive cells angular instead of rounded outlines. It is, moreover, hard to determine always to which egg they belong. There is no increase in their number from the beginning.

The next step is the formation of the egg membrane and the degeneration of the nutritive cells. From the very slight development of the follicular cells it might be inferred that they take no part in the formation of the envelope of the egg. This is found to be true, and the process of formation by the egg itself is as follows: When the full size is reached the outer surface of the yolk undergoes a process of disintegration. Large yolk granules are broken up into smaller ones, and these in turn are finally transformed into a thin protoplasmic layer which shows clearly its network structure. This mesh-work gradually becomes obliterated, first on the outside and later farther in, as it were by condensation, until it shows an almost homogeneous structure outside preserving its fibrillar character inside (Fig. 12, *e.m.*). From the beginning, it stains deeply with Lyon's blue, a fact true of the envelope of the egg in later stages. Its formation is almost simultaneous over the whole surface, excepting in the region of the nutritive cells, where it appears a little later, a fact observed by Korschelt ('89a) in other insects. Fig. 12, *e.m.*, shows the envelope in an advanced state. The hardened homogeneous exterior already exists and the nutritive cells are separated from the ovum. They have shrunk in size; the cytoplasm has almost entirely

disappeared; the chromosomes have lost their stellate structure and are fused into irregular masses. The whole cell appears as a mass of deeply stained chromatin. The final stage when the egg envelope is fully formed and the nutritive cells have entirely disappeared is reached before the egg is laid, no sign of any outside cells being present in the last stages of the ovarian egg.

It is clear from the foregoing description that the envelope formed is not a chorion in the true sense of the word. It is a "Dottermembran," "Dotterhaut," such as is found among crustaceans, and is described in many myriapods. Schmidt ('95) in describing *Pauropus* designates the egg envelope as a "Dottermembran," and gives a process of development closely similar to that described for *Anurida*. Among insects the outer envelope is without exception, so far as determined, a true chorion formed by the follicular cells of the egg tube. The vitelline membrane which appears later is, however, a true "Dotterhaut" formed by the surface of the egg. This rule does not hold with *Anurida*; here the ovarian membrane is formed by the egg itself and is a "Dotterhaut," as also is the vitelline membrane which is formed after the egg is laid. Hence, in this respect *Anurida* resembles myriapods and crustaceans rather than insects.

It is hardly necessary to say that the paired structure of the ovary is entirely obscured in the mature stages of egg development. Eggs are crowded back into the short unpaired part and fill the body down to the oviduct. Ova are crowded above and below the alimentary tract, and no available space in the body is unused.

Ovaries after egg laying are irregular and collapsed. The only germinal cells present are in the mass of germinal epithelium. There are no large ova or traces of nutritive cells and cell masses. Whether the animals survive and lay again in the following summer or whether they die after one season has not been determined. The only evidence on the matter is this: towards the end of the summer the adult animals become scarcer, although eggs are still abundant. In the early spring, the beginning of April, there are none to be found anywhere,

while all the earliest animals to appear in the summer are smaller than those found later in the season, and some of them exceedingly small. It might be inferred from this that the old animals die and the young ones remain in the sand where they go when first hatched. This accounts for their absence in early spring and the presence of very small ones among those first found in the summer. The largest ones would be those first hatched in the preceding summer and the smallest the last; the latter have grown very little, and their chief advance from the newly hatched condition is in pigmentation. The absence of any dead bodies of the adults in the fall is easily accounted for by their exposure to the tides and consequent removal.

One more point was considered in connection with the ovarian egg; that is the existence of a micropyle. No evidence of such a structure was found; if it exists it must be extremely small. No special cells can be found taking part in its formation; the contrary is true of many of the Orthoptera, and still more so of the complicated micropyles found in Diptera, Lepidoptera, and others. Whether the places occupied by the nutritive cells would have anything to do with such organs was not decided; it seems more probable that they have some bearing on the connection of the eggs with one another. As shown in Fig. 15, *a* and *b*, the eggs are united firmly where they come into contact with each other by a thickened plate of the envelope; this is formed in the ovary, as there are no glands in the oviduct to serve any such purpose. These plates can be seen formed in unlaid eggs. As shown in Fig. 11, the follicle does not divide the eggs from each other where the nutritive cells are, and it appears that at this point the membranes are later brought into contact and firmly cemented together. Hence, the eggs must pass from the ovary in a solid, more or less irregular mass. In several cases an egg was found in the anterior part of an ovary after egg laying in an advanced state of degeneration, and the inference is that it failed to be attached to the rest of the mass and hence was not laid.



*Maturation.*

As has already been seen, the germinal vesicle disappears early in development, before any yolk has been formed. The nuclear wall is lost and the chromosomes are too small for recognition in the greatly increased mass of cytoplasm. From this time onward no sign can be found of the nucleus. The most careful search fails to reveal anything even in the mature ovum just before laying. No polar body spindle has been found, and the first reorganization of the nucleus making it visible is found in the section of the egg shown in Pl. XXI, Fig. 25, where the polar bodies have just been given off and the female pronucleus is returning to the centre. This egg has been laid some little time, and the male pronucleus is already at the centre awaiting the return of the egg nucleus.

Naturally *Anurida* is a most unfavorable form for the study of nuclear changes. Nothing determinate has been observed. Gradual shrinkage is the description best fitting the only change observable. The changes taking place in the ova, shown at *a.o.* in Figs. 10, 11, and 12, are curious, and perhaps show what occur in the others. Up to a certain stage it is impossible to tell whether a cell will develop to an egg or one of these abortive ova. Fig. 9, *o.*, may be either. There is the same reduction in the amount of chromatin, but instead of ultimately losing the nuclear membrane this swells up, enlarges, and forms a large germinal vesicle almost obliterating the cell body (Figs. 10, 13, *a.o.*). The chromosomes enlarge slightly, and, moving from the periphery, definitely arrange themselves in groups of four. This occurs at about the time of maximum yolk development, degeneration and absorption following soon after. A possible explanation of their existence may be that, although a certain number of germinal cells assume the characters distinctive of ova, still there is not nutriment enough to mature them all; hence, some are brought to a standstill in their onward progress by lack of food and undergo the degenerative hypertrophy spoken of before. The changes occurring in the nuclei of these cells may then be, in a measure, indicative of those occurring in the much smaller germinal vesicles,



and the number of chromosomes may fairly be assumed to be the same, eight, typical of insect ova.

It is common among insects for the nucleus to be so small that it can only be distinguished with great difficulty,—so much so that for a long time it was said to have disappeared altogether, and the female pronucleus appearing later was described as a new structure. This has, however, been proved incorrect, and several writers—Wheeler ('89) in *Blatta* and *Doryphora*, Blochmann ('84), Will ('85), and others—have described fully many complicated changes taking place in the germinal vesicle. It is evident from their descriptions that these changes occur later than do the corresponding ones in *Anurida*. In both *Blatta* and others the germinal vesicle remains intact until yolk formation has been almost completed; then the curious phenomena take place which render the nucleus so small; this is followed by the formation of the first polar body spindle, in which condition the egg is laid. It would seem rational to infer from this that the nucleus in *Anurida* exercises less control over the egg during the formation of the yolk than in the higher *Insecta*; perhaps this duty is performed more completely by the nutritive cells. No sign has been found of the polar body spindle nor any indication of the position of the nucleus until a much later stage than in other insects. In this respect *Anurida* resembles the myriapods, in many of which the germinal vesicle disappears early in ovarian development and is not seen even in the last stages. Lubbock ('61) says that in both *Glomeris* and *Julus* no trace of the germinal vesicle is ever seen in mature eggs. Zograff ('90) makes the same statement regarding the ripe ova of two species of *Geophilus*. Schmidt ('95), on the other hand, in describing the process in *Paupopus*, states that all through the period of yolk formation the germinal vesicle undergoes no change; it remains as distinct as in the younger eggs.

#### *Comparative and General.*

Passing from this brief description of the ovarian structure and oögenesis to a general consideration of the conditions of the ovary and a comparison with other forms, it is found that

Anurida differs markedly from the typical insect. The chief structural points of difference are the following:

- (1) Simple paired ovaries.
- (2) Absence of ovarioles.
- (3) Arrangement of eggs in follicles.
- (4) Presence of the germinal epithelium in two masses at the hinder end of the body.
- (5) Absence of a coiled complex oviduct in which membranes, etc., are secreted.

One striking point of resemblance will be considered first; that is, the cephalic elongation shown in Fig. 1, *c.el.* This undoubtedly suggests the "Endfaden" of the ovariole, but one or two points are sufficient to show that the exact resemblance is superficial, though an analogy of function may exist. Taking the ovarioles in *Blatta* as typical, they are found to be numerous slender tubes, containing maturing eggs at their caudal ends, a mass of germinal cells in the middle; while the cephalic ends are prolonged into fine threads, which, uniting with threads from the other ovarioles of the same ovary, pass forward as a single strand. Heymons ('91) discusses the various theories regarding this strand, which passes cephalad and disappears or becomes attached near the heart. Müller, Wagner, and Blanchard say it is nutritive in function, an inference from its direct connection with the heart. Leydig considered that it is a membrane made of peritoneal tissue, and that it is homologous with the ovary, being formed of similar epithelial cells. Stein, Kramer, and Dufour consider the "Endfaden" simply ligamentous in function, a suspensory ligament for keeping the ovarioles in place. Heymons ('91) agrees with this view. Korschelt ('86) says that in *Dytiscus marginalis* the cell boundaries in the "Endfaden" are lost, the nuclei decrease in size away from the ovary and show evidences of undergoing indirect division, which in itself sets the cells apart from any reproductive function.

It has been satisfactorily demonstrated by Korschelt and others that the "Endfaden" is exclusively ligamentous in function; it is formed by the union of the cephalic elongations of the ovarioles; its cells have no reproductive characters. It

is clear that the cephalic elongation in *Anurida* is also of the same nature; it serves to keep the ovaries attached to the anterior body wall. Its relation, however, to the germinal epithelium is entirely different; it is entirely separate and not directly continuous, as in other cases. The position of the germinal epithelium in the third and fourth abdominal segments distinctly cuts it off from any direct connection with a strand that originates in the first abdominal segment, apparently as a simple continuation of the wall of the ovary, the cells of which the two are composed being closely similar (Figs. 4, 5, *ov.*). In *Anurida* as in *Dytiscus*, according to Korschelt ('86), the cell outlines become indistinct and are in some places lost, but particularly near the ovary the cells are arranged with almost a lumen in the centre as in a duct.

The great discrepancies between the form of the ovary in *Anurida* and that shown by *Blatta* or any other typical insect



FIG. I.

led to a comparative study of the gross structure of as many forms of insect ovaries as could be reached in literature or otherwise. As regards typical forms, Lubbock ('59) says the number of egg tubes in insect ovaries varies from two to two hundred (queen bees). In no case could records be found where the number was less than two. Even in the ant workers, according to Bickford ('95), where the reproductive organs are practically abortive, the number varies from two to fifteen, while in the queen, the fertile female, there are forty-five ovarioles in each ovary. Starting among the Orthoptera it is found in the *Blatta* ovary that the average number of tubes is about twenty. They are arranged so as to open at very slight differences of level into oviducts, which in turn unite to form the common oviduct leading to the exterior. Fig. I in the text is copied from a plate given by Dufour

('28). It represents the ovary of *Labidura riparia*, a forficulid. Here there are five ovarioles to each ovary. They are figured as opening into the oviduct at different levels, making it possible to designate the ovariole as the first or fifth in a series.

This fact is true of *Blatta* also, as shown by different authorities. The cephalic ends are united, but there is no "Endfaden"; it is quite possibly present, however. Fig. II is a copy of the same author's representation of the ovary of *Forficula auricularia*. Here, as Lubbock ('59) says, the ovaries consist of numerous very short tubes, three rows in each ovary. These open into a large tube which passes back into an enlarged chamber. The cephalic end shows a curious elongation that clearly unites with one of a similar nature from the other ovary (Fig. III); how these finally end is not figured nor described.

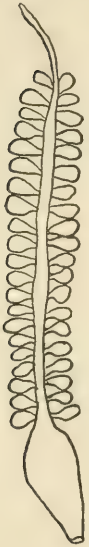


FIG. II.

Passing now to the Thysanura, an interesting and suggestive series can be arranged. According to Grassi ('88), each of the ovaries of *Machilis* consists of seven short egg tubes. These open into two straight tubes running the length of the body (Fig. IV); according to Oudemans ('87),

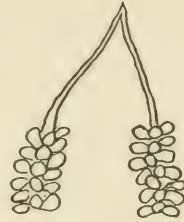


FIG. III.

each of these tubes is continued into a separate oviduct, and both of these lead into a common opening without union. There is in this stage nothing metameric about the arrangement of the tubules. The germinal epithelium is at the ends of the tubes, but there is no evidence of the "Endfaden" as developed in higher forms of the insect ovary; Oudemans ('87) distinctly recognizes its absence. The conditions found in *Japyx* are somewhat similar; here there are egg tubes shorter than in *Machilis*, and arranged metamERICALLY in the first seven abdominal segments (Fig. V); the "Endfaden" is also absent. *Campodea*, shown in Fig. VI after Grassi ('88), has a distinctly different structure from the two preceding Thysanura. Each of the two ovaries has the appearance of consisting of a single ovariole. This is, however, much longer than in the typical



FIG. IV.



insect, as it reaches the whole length of the abdomen; the germinal epithelium is at the anterior end just as in the typical case. Grassi ('88) describes and figures the ovary of *Lepisma* as consisting of five tubules arranged on the same plan as in *Machilis* and *Japyx*, differing in being distinctly metameric in the young individual but not in the adult. *Petrobius maritimus*



FIG. V.

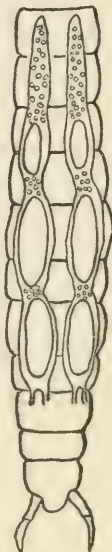


FIG. VI.

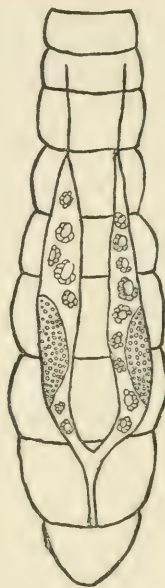


FIG. VII.

is described by Lubbock ('61) as having seven short ovarian tubes on each side of the abdomen; these tubes lie *above* the intestine.

Passing to Fig. VII, where the conditions found in *Anurida* are somewhat diagrammatically represented, there is evidently a great divergence shown from the types already studied. It cannot be directly harmonized with any, agreeing as little with *Campodea* as any other. The presence of two cephalic elongations is the chief point of resemblance. These, however, are not connected in any direct way with the germinal epithelium; they are apparently thin continuations of the ovarian wall. *Anurida*, then, stands quite distinct in structure of the female



reproductive organs from any pterygote or even apterygote insect thus far studied. Passing down the line of tracheates the nearest related forms are among the myriapods, and here are found possible explanations for some of the peculiarities noted.

The myriapods, as a group, present so many distinct types in the structure of the reproductive organs that the validity of the group has been reasonably called into question. Without going into any discussion of the matter, a few points of comparison may be taken from among the different groups. According to Vogt and Yung ('90), the ovaries of the Chilopoda, taking *Lithobius* as a type, are large, unpaired, flattened, irregular masses of eggs opening by an oviduct at the hinder end of the body. They lie in the posterior part of the body *over* the alimentary canal and below the heart, while the single unpaired opening is ventral. The eggs develop irregularly in the ovary, which is rounded and widest at its cephalic end with no trace of an "Endfaden." Sections made of a just-born specimen of *Scolopendra complanata* show a median unpaired ovary lying *over* the alimentary canal and attached to it, appearing as if the cells giving rise to the ovary originated in the outer wall of the intestine.

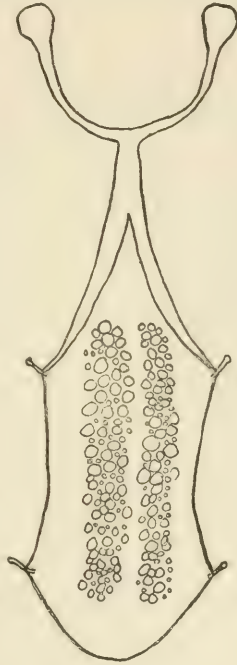


FIG. VIII.

Fig. VIII is taken reduced from Fabre ('55), whose work on myriapod structure is still considered authoritative with the additional value of recent confirmation by Vom Rath ('90). This figure shows the ovarian sac of *Glomeris marginata*, a chilognath. The first impression is that this ovary is also unpaired, but, as is common with this group, this is a derived condition; the two ovaries were primitively distinct, but a fusion of their walls took place on the median line, forming a single sac.

The ovarian sac lies below the alimentary canal, between it and the nervous system; the openings are paired and anterior,

between the second and third body segments. Fabre ('55) and others describe the ova as developing exclusively on the ventral surface of the ovarian sac, which reaches back into the hind body segments. Two strings of ovules, "placentaires," extend throughout the length of the ovary, united to the membrane of the sac. Each egg is enclosed in a separate follicle, which eventually breaks, allowing the eggs to fall into the unpaired ovary, formed by the fusion of two primitively distinct sacs. Heathcote ('88) describes the ovary of the just-hatched *Julus terrestris* as an unpaired sac enclosing a double line of ovules; earlier there were two distinct sacs; a fusion followed. According to Schmidt ('94, '95), who agrees with Grassi ('86), the ovaries in Scolopendrella are paired, with paired anterior outlets opening upon the fourth segment. Each ovum is enclosed in a distinct follicle. Schmidt ('95) and Kenyon ('95) both agree in their description of Pauropus, the lowest form of myriapod; they represent it as having an ovary of the typical diplopod type. It consists of a large unpaired sac lying on the median line below the gut, crowded with ova, the largest of which are forced forward and sideways, leaving a central and posterior mass of small ova. A small unpaired oviduct opens in the third segment a little to the right of the mid-ventral line. No evidence of double strings of these ova has been observed, but only mature or nearly mature females were studied. The ova are inclosed in follicles within the ovarian sac.

Briefly summarizing the conditions found in the myriapods: in the Chilognatha the ovary is an unpaired sac, the germinal epithelium is placed chiefly at the hinder end of the body, and the developing ova pass forward through the successive segments as they ripen. The oviducts begin usually in about the fourth or fifth segment and open in paired outlets on the second or third. It is evident that a paired condition primitively existed, and that the unpaired ovary is the result of fusion of two sacs. The primitively paired condition is still indicated by the development of ova in the young animal and by the paired openings. In the Symphyla, Scolopendrella, the paired condition persists in the adult, the opening appearing on the fourth body segment. The Pauropoda show unpaired ovaries with a cephalic, asym-

metrical opening in the third body segment, and no traces of paired origin unless the eccentric opening may be such. In the Chilopoda, paired conditions are lost; no traces of them have been found; the opening is posterior and unpaired also. One more point of interest in connection with the structure of the myriapod ovary has been advanced by Lubbock ('61). He makes a fundamental distinction between the principles of follicle formation in insects and myriapods. In the latter the follicle projects *into* the ovary, while in the former it projects *from* the ovary. The importance of this distinction may not be great, but the comparisons already started between myriapod and hexapod ovaries may explain it. Comparing Figs. I-VIII the following line of development can be traced. Beginning the series with the generalized condition shown in Fig. VIII, Glomeris, and disregarding the anterior opening, Anurida is easily derived by a slightly greater localization of the germinal epithelium and non-fusion of the ovarian sacs. Japyx (Fig. V) can be derived directly from Glomeris by localizing the points of origin of ova still more sharply, and, arranging the ova in strings, by retaining a connection between the maturing eggs instead of dropping them at once into the ovarian sac, posterior unpaired openings having succeeded paired anterior ones. Machilis (Fig. IV) is the result of a condensation of the conditions started in Japyx; the tubules are elongated and crowded together. From the method of development, the germinal epithelium is at the ends of the tubules. *Labidura riparia* shows a further concentration of tubules, which in this case number only five. To gain the most highly developed hexapod ovary, it is only necessary to increase the number of tubules, which could be done primarily or secondarily. Heymons ('91), in his studies on Blatta, shows the process of origin of the numerous tubules found in the adult; from a mass of undifferentiated cells arise by rearrangement the "Endfaden" of each ovariole, as well as the common one binding the tubes together.

The conditions found in *Forficula auricularia* (Figs. II, III) form an interesting and instructive phase in the line of development. Here, according to Fabre and Lubbock, the ovary consists of very numerous short tubes, perhaps each containing a

single egg, opening successively into the ovary. This typically illustrates the difference in the position of the myriapod follicle and that of the insect, and shows the possible method of origin of the numerous tubules by progressive localization from the conditions shown in *Glomeris* (Fig. VIII). Another interesting point is the evidence of a cephalic elongation, which, in the case of *Forficula*, unites with one from the opposite side (Fig. III). Its anterior attachment was not determined.

The step from *Glomeris* to *Anurida* is short and clear; *Anurida* still retains the ova developing in intra-ovarian follicles, and a reduction of the germinal epithelium to a mass in the third and fourth abdominal segments is the only change excepting for the altered position of the outlet. Still further, the arrangement of the ova in strings is possibly an indication of the development of egg tubules similar to those found in the higher *Apterygota*.

Supposing this to be true, it is evident that the morphological value of the egg tube in *Machilis* and the other thysanurans is not necessarily different from that of the higher *Pterygotes*. Increase in number simply means arrangement in a greater number of strings, a device for accommodating more germ cells compactly in an individual. Heymons' study on *Blatta* strongly seconds this view. This aspect does not support the opinions held in regard to the primary metamerism of the egg tubes in *Japyx*. If the insect ovary came through any such series, headed by such structure as is shown in *Glomeris*, it is evident that metamerism, if it existed in yet earlier forms, has been obliterated; and such evidences as are seen in *Japyx* are either reversions or due to secondary development.

Returning once more to the series of text figures (I-VIII), there is an evident lack among the Thysanura of anything corresponding to the "Endfaden." With the exception of *Anurida*, there are none that show a trace of such a structure. This absence may perhaps be accounted for by the following facts. The ovaries of *Machilis*, *Japyx*, and *Campodea* are comparatively simple; in the first two the ovarioles are short and small and, especially in *Japyx*, spread throughout the body. There is not the mechanical demand for an anterior suspensory ligament



that arises on the increase in number and length of tubes found, for example, in *Blatta*. In origin the cells of the "Endfaden" arise from the same source as those of the ovary, and it can be considered as simply elongations of certain parts. On this basis it is difficult to see why two forms, preserving as simple an ovarian structure as that found in *Anurida* and *Forficula*, both show an anterior elongation. A possible interpretation will lie along another line. The elongation in *Forficula* is totally distinct from the germinal epithelium, which lies at the free ends of the short tubes. The two parts unite on the middle line (Fig. III). In *Anurida* the elongations are also distinct, and from their union with the fat body in the thoracic segments evidently serve the purpose of suspensory attachments. Cross-sections of these chords near the ovary show in some cases a distinct lumen in the middle of them. In some cases this persists for some distance, giving the thread the structure of a fine tube. In the embryo this part of the ovary is very striking and bends over distinctly towards the ventral wall of the first abdominal segment (Fig. 58). It appears long before any duct at the hinder end has begun to develop. Its position, structure, and relations in the adult ovary and its early development and peculiar relations in the embryo all strongly suggest a possible connection with the oviducts of the chilognath or symphyliid. Change of function would now account for its relations to the second thoracic segment of the adult, but its embryonic relations suggest that it once was connected with the first abdominal segment, which bears the collophore. A suggestion might be made in regard to this problematic organ, which has been considered in so many different aspects. Wheeler ('90), following Graber ('89) and Carrière, gives these views at length, and it is only necessary to say that whichever of the three functions it serves,—of gills, sense organs, or glands,—it is undoubtedly a pair of fused appendages. In the Symphyla, which, according to Haase ('86), are nearly related to the hexapod ancestor, the genital opening lies in the median line between the fourth pair of legs (Haase, '89, Fig. 1). A study of the genital openings of *Polydesmus*, *Craspedosoma*, and other chilognaths, as given by Fabre ('55),



shows that the appendages on the segment bearing the genital opening have been more or less changed. Histologically, the condition of these modified appendages is given as being highly vascular, a description closely agreeing with the structure of the collophore in *Anurida*. On these grounds it is then possible that the collophore in *Anurida* is a relic of a former anterior opening for the reproductive organs, and that the cephalic elongation is a trace of the former duct. The exact present function of the collophore has long been a source of much conjecture, and still remains in doubt.

Fernald ('90, p. 45) summarizes the views held as to the possible present function of the ventral tube in the *Collembola*; two writers think it a genital organ, but its equal development in the male and female argues against this. Haase considers it a blood gill, a function he assigns to all the rest of the similar abdominal appendages found in the *Symphyla*, while it is said also to be a gland for the secretion of an adhesive mixture. Any of these functions may have been acquired since its original function was lost, but the evidence given above and its position on the fourth body segment—the same as that of symphyloid genital opening—give probability at least to the view here advanced of its primitive function. Kenyon ('95) gives a table of the appendages and their homologies in the various groups of arthropods, in which he considers the fourth segment of the *Symphyla* as the homologue of the third in hexapods, but upon what basis is not clear. Haase, Wheeler, and Grassi agree in thinking the homology of the fourth with the first abdominal correct. This view is still further supported by the suggestion of Grassi that the symphyloid ancestor of the *Insecta* had paired genital apertures at the hinder end of the body and also another pair between the third and fourth pairs of legs. That the genital openings among the hexapods are not very fixed in position is evident from the variety of conditions found in the different members of the group. Wheeler ('93) shows that the openings of the male and female differ, and an actual movement takes place in the former from the tenth to the ninth abdominal segment during embryonic life.

*Nutritive Cells.*

The possession of nutritive cells is a character widespread among the Insecta. In the majority of cases, ova are associated with a definite number of cells that assist to a greater or less degree in yolk formation and in the general growth of the ova. Korschelt ('86) divides insect ovaries into two classes, according to whether they possess these accessory cells in the form of a nutritive chamber or otherwise. Sometimes they are arranged in separate chambers lying between the eggs, and sometimes in a terminal chamber. Many curious forms are described by different writers. Claus ('64), using *Coccus* and *Aspidiotus*, found "yolk strings" leading from nutritive cells to the egg, in some cases passing through the chamber of an undeveloped egg. The same structure, a "Dottergang," is described by Wielowiejski ('85) in *Pyrrhocoris*. Sometimes the nutritive cells are arranged in a highly developed follicle and absent in any other form. Korschelt ('86) figures *Vanessa urticaria* as possessing a few such cells, closely applied to one surface of the egg, an arrangement resembling that found in *Anurida*. Among the Apterygota, Grassi ('88) figures *Campodea* alone as having anything similar to nutritive cells; here, as shown (Fig. VI), they are interpolated in chambers between the eggs. He expressly states, however, that the nutritive cells are *not* homologous with those of the Insecta; why he does not say, unless he wishes to consider the ovary as not the morphological equivalent of the ovariole of the higher Insecta. In the thysanuran (*Tomoceras* sp.?) nutritive cells were found closely similar to those described for *Anurida*, but smaller and arranged more distinctly as a follicle and present in larger numbers (Fig. 14). In *Anurida*, as before described, each egg is associated with its string of nutritive cells, varying in number from five to eight. Will ('84, '85) gives the results of his studies, and presents in full his idea of the compound origin of an egg, and the formation by the egg of some of the follicle cells. As he has, however, changed some of his recent statements of the case, these studies will not be mentioned in detail.

Among the myriapods, very little trace can be found of anything resembling nutritive cells; in part they are absent, and in part too little histological study has been made of these forms. However, Schmidt ('95) describes in *Paupopus* a peculiar process that he interprets as the nutrition of eggs by other eggs. Ova have no definite arrangement in the hinder end of the ovary. Some cells of the germinal mass grow in size and gain yolk at the expense of the surrounding ova, which cease growing and finally degenerate. In *Scolopendrella* he describes an even more astonishing process in which he considers the follicle cells to be opposed to the egg cells, and *vice versa*. Follicle cells have the power of migrating into the egg cells. This migration is followed by one of two alternatives: either the egg absorbs the follicle cells or the follicle cells overpower the egg cell as phagocytes, destroying it, and afterwards wander out to become the follicle cells of a stronger egg cell. A similar case is mentioned by Weismann in *Leptodora*, not, however, so phagocytic in character. Yolk is formed after this battle is over, and all changes in the germinal vesicle are subsequent to this process. No mention of any such relation can be found among the diplopods or chilognaths. Stuhlmann ('86) figures eggs of *Julus* *sp.* ? and of *Glomeris marginata*, in neither of which are there any traces of nutritive cells.

Passing from the arthropods, Wheeler ('96) describes many forms of "Nährzellen," or accessory cells, in different groups of "worms." The ovum of *Myzostoma* is accompanied by two accessory cells, which gradually lose their individuality as the ovum matures. *Ophryotrocha*, described by Braem ('93) and Korschelt, sheds its ova into the body cavity with one accessory cell. The ovum of *Tomopteris* is accompanied by seven smaller cells, while in *Diopatra* it bears two long strings of cells attached laterally, which strings fall off before the ovum is mature. Numerous other cases are given, but these are enough to show the parallelism of development and resemblance to those found in *Anurida*. In the latter case the ovum accompanied by a certain number of cells is pushed away from the germinal mass of cells into the ovary, the cavity of which is the homologue in the hexapod of the annelid body

cavity. Here they are pushed forward by those developing behind, and are later inclosed in follicles made from the wall of the ovary. The difference between this follicle and that usually found in the hexapod ovary is in its direct origin from the ovarian wall, not from certain special cells in the germinal epithelium.

No further light is thrown by *Anurida* on the exact part taken by these accessory cells in the development of the ovum. No support is given, however, to Will's idea of the direct transformation of follicle cells into the yolk (Will, '84). Certain changes occurring in the nutritive cells previous to yolk formation and the disappearance of the material shown just preceding and during the early stages of the growth of yolk supports the view held by Blochmann ('84), Schütz ('82), and others that the nutritive cells secrete a ferment, the precursor of the yolk; this passes from the cells into the ovum and then the yolk appears. This material is probably formed also in the ovum itself. The persistence of these cells up to the time of maturity of the ovum till all the yolk is formed, their undiminished size up to this point, and their rapid degeneration afterwards, indicate an active relation between them and the egg.

The existence of lines of communication between the accessory cells and the ovum, such as was seen by Claus ('64) and Wielowijski ('85), is evidence of a higher degree of differentiation; probably the material passed into the ovum in cases where there is a visible connection is more highly elaborated by the nutritive cells and requires less adaptation by the egg. As far as the origin of these cells is concerned, it is generally agreed that they arise from undifferentiated germ cells; it may be, perhaps, owing to a peripheral position on the germinal epithelium that more nutrition reaches them, or some other less evident cause may prevail. One thing is clear, that an added supply of chromatin is one of the first changes; from an originally small chromosome the mass increases by branching and spreading. Fine threads reach out in every direction as if to offer a larger area for contact with the nuclear plasma. It finally assumes in *Anurida* a strikingly stellate structure (Figs. 10, 11, 13).



Passing now to the early changes in the nutritive cells, a consideration of that peculiar structure known as the yolk nucleus is necessary.

The "yolk nucleus" is a term of varied application and, consequently, great indefiniteness. Two distinct classes of structures have been designated by this name: (1) embryonic cells concerned with yolk absorption whose origin is assigned variously to entoderm, mesoderm, superfluous spermatozoa, or the germinal vesicle, and whose fate is said to be absorption or transformation into the midgut epithelium; (2) all those structures appearing in the ovarian egg which can have but one of two possible origins: nucleus or cytoplasm of the cell itself, and whose existence may cease before the yolk appears in the egg or may be continued into embryonic life.

It is on the second of these classes that the facts observed in *Anurida* have a bearing. The peculiar "blue caps" that appear at a certain stage in the nutritive cells can have no other homology than the "yolk nucleus," the "Dotterkern" or "Nebenkern" of some authors.

Hubbard ('94) gives a list of groups in which the yolk nucleus has been observed to occur. This list includes all the classes of arthropods, cnidarians, nematodes, *Sagitta*, lamellibranchs, gasteropods, and all the vertebrate groups. The nucleus is found in eggs associated with nutritive cells and those without. Stuhlmann ('86) described it in *Bombus*, *Vespa*, *Trogus*, *Pimpla*, and *Bauchus*, varying in form from a diffuse peripheral mass to a localized spot. He, however, reduces the method of origin in all the Hymenoptera to one type, — that of a small concretion appearing close to or in the near vicinity of the germinal vesicle. This mass wanders away, forms a peripheral layer, collects at one pole or is scattered in several diffuse masses. Stuhlmann never satisfied himself of its nuclear origin as Balbiani ('83) had in *Geophilus* and Will ('84) in the frog. In all cases it is in very young ova that it appears; it becomes invisible on the formation of yolk. No mention has been made of the existence of the yolk nucleus in any other of the forms of insects. The appearance in *Anurida* of this blue cap in the nutritive cells declares the presence of this curious adjunct of



the ovum. It is true that no traces have been seen in the ovum itself, but the position close to the nucleus of the nutritive cells at the time just previous to yolk formation and its subsequent disappearance are all indications of its homology with the "yolk nucleus" of the second class.

Among the myriapods this body has been extensively observed. Balbiani ('83) and Zograff ('90) both report a yolk nucleus present in the small ova of the chilognath *Geophilus*. Balbiani figures and describes it as originating from the nucleus and in part forming follicle cells and in part the undoubted yolk nucleus, which in this case in its greatest development has a radiate structure suggesting the aster of cleavage spindles. It disappears while the ova are still very young. Heathcote observes the absence of a complex yolk nucleus in *Julus* such as was described for *Geophilus*. Lubbock ('61), describing the eggs of *Julus*, notes the absence of the vitelligenous bodies described for *Glomeris*, but notes the presence of a small body in eggs of an intermediate size that he compares to the laminated body of spiders' eggs; evidently it is a yolk nucleus. It is absent in smaller and larger eggs. Lubbock describes vitelligenous bodies as present in the egg follicles of *Glomeris*, but distinguishes them from yolk nuclei. Stuhlmann ('86), however, figures part of the ovary of *Glomeris* in which yolk nuclei are evidently present, and, from his descriptions and figures, more closely resemble those found in *Anurida* than any others hitherto described. Kenyon ('95) describes a small body near the nucleus in the young ova of *Pauropus* which disappears in older stages to be followed by yolk spherules, which is evidently the same structure, although not called so by him. Schmidt ('95) describes in *Scolopendrella* a small body inside of the egg cell staining as deeply as the nucleus, which he calls a migrated follicle cell, but which is quite possibly the yolk nucleus. Stuhlmann ('86) figures a large yolk nucleus present in the ova of *Peripatus edwardsii* which, strangely enough, persists late in the egg's history, even after fertilization. It is not necessary to enumerate any more forms of animals in which this structure appears; it can be said to occur among the remaining invertebrates and the vertebrates, with many variations

in its history. Usually it is present only for a short time just before yolk formation.

There can be no doubt that the yolk nucleus has an important part to play in the developing egg, and that its function concerns the formation of yolk, as agreed by Stuhlmann and Schütz. Balbiani, Sabatier, and Jatta formerly held a view regarding it as a fertilizing element, precursor of the sperm cell, but this has long been abandoned. A question naturally arises as to the origin of this necessary body, for which some name ought to be found excluding the term nucleus with which it has nothing in common, or very little, even if its nuclear origin were demonstrated. Balbiani, working chiefly among arachnids, where the yolk nucleus is largely developed, suggested its homology with the centrosome of the spermatozoan and somatic cells. Agreeing with Boveri that the centrosome has no part to play in the female cell, he considers the yolk nucleus as a case of a true hypertrophy of degeneration. It is easily supposable that in the process of degeneration some ferment should be originated useful in yolk formation; hence its preservation under a new form. Its appearance in the ovum just previous to the beginning of growth may indicate the fact that the period after the last division has taken place is when degeneration of the centrosome sets in, accompanied by its useful hypertrophy. Balbiani's figure of *Geophilus* shows a strikingly radiate structure in the "yolk nucleus."

The late persistence of this body in the egg of *Peripatus edwardsii* is perhaps explicable on these grounds. This species of *Peripatus* is viviparous, and the appearance of intra-uterine development in this form reduced the amount of yolk needed. This was consequently decreased, but as yet the reaction has not included the formative material; this is still formed, perhaps, in unreduced quantities, and hence remains not transformed into yolk. It has not yet responded to the changed conditions which require a smaller amount of yolk.

It is interesting to note that the yolk nucleus and its origin bring up the much-disputed question as to the origin of the centrosome. Balbiani's arguments demanding the origin of the yolk nucleus from the nucleus naturally carry back the

centrosome to the same source. On the other hand, Stuhlmann and others fail to determine the nuclear origin, their evidence pointing to the cytoplasmic nature of the centrosome. As far as *Anurida* is concerned, there is no clear evidence of a nuclear origin. The yolk nucleus always appears in the cytoplasm close to the nucleus and on one side, but there is no reason to think that it originates more from one than from the other: it has more the appearance of being the result of the joint activities of both. At least, this question cannot be settled without determining the greater one, the origin of the centrosome, if such an homology as has been suggested be accepted.

#### *Unsegmented Ovum.*

The eggs of *Anurida* when freshly laid are easily recognized by their peculiar light yellow color and smooth, shining surface. They are found fastened together in masses, the number of eggs in a mass varying from a few, five or six, to at most fifteen or twenty; each egg is about 0.27 mm. in diameter. These masses are sometimes arranged irregularly, the eggs being simply fastened together in a pile, as shown in Fig. 15, *a*; or there may be a definite form to the mass, owing to the position of the eggs in two rows in which the eggs alternate with each other (Fig. 15, *b*). Very frequently in the central part of an ovum can be recognized a lighter spot; this shows the position of the central mass of protoplasm in which the cleavage nucleus is placed. The egg membrane is closely adherent to the surface of the egg in these early stages, but before cleavage takes place a distinct space has appeared between the membrane and the egg. No further points of differentiation are visible from the outside, but the internal structure is marked. Pl. XXII, Fig. 30, shows a section through an ovum having the external characters described above. The outer egg envelope and the vitelline membrane, which is very thin but distinctly formed by this time, are both omitted in the figure. The egg itself is seen to be formed at this time of a large central mass of protoplasm (Fig. 30, *c.p.*) with many radiating strands (*r.*), which branch as they pass to the periphery, and in most cases finally connect with a thin protoplasmic layer

that surrounds the outer surface of the egg. Between the strands of the meshwork formed by the radiating protoplasm lie the numerous yolk bodies (*y.*), which vary much in size. In the central mass of protoplasm, either central or slightly to one side, there was usually present a rather large pear-shaped nucleus which proved to be the male pronucleus (*m.pr.*); after entering the egg it has assumed its permanent central position and is awaiting the return of the female pronucleus from the periphery. In many eggs there was a smaller mass of protoplasm on or near the outer edge, as is shown in Fig. 30 at *p.i.* In this is found the female pronucleus, the egg nucleus, for the first time recognizable since its disappearance before yolk formation began. Fig. 25 shows an enlarged view of this mass as seen on the periphery. Large yolk bodies (*y.*) are recognized imbedded in a protoplasmic mass that extends to the surface. At *f.pr.* can be seen the extremely small female pronucleus returning to the centre of the egg to meet the sperm nucleus that is already there. *P.b.* shows the small and incompletely separated polar bodies that have been given off by the egg nucleus, thus completing its reduction to the female pronucleus. Fig. 26 shows the two polar bodies at *p.b.1* and *p.b.2*; as shown, the first polar body is again dividing, giving the typical number of three. Somewhere in the protoplasm is the female pronucleus, but not clearly outlined. The polar bodies never completely separate from the egg and never clearly protrude from the surface of the egg, but remain flattened down and are eventually absorbed into it. No sign has been found of the polar body spindles; the egg nucleus preserves its invisible condition in spite of the most careful searching, the first reappearance being in the form of the female pronucleus and polar bodies, as shown in Fig. 25. With the gradual passage of the female pronucleus towards the centre of the egg there is a withdrawal of the strands of protoplasm in the same direction, so that the central mass gradually increases in size until at the beginning of cleavage it is plainly visible in a fresh, whole egg as a large, lighter, central spot. In Fig. 27 is shown a part of this mass, and in it at the centre the two pronuclei,—the larger, the male pronucleus, pear-shaped,



and the smaller, the female pronucleus, a round ellipse. Some of the eggs contain very small central masses; several showed, instead of any central mass, a small one about halfway to the centre, distinctly containing a small nucleus. These facts point to the following interpretation: when the sperm cell enters the egg (just when this occurs was not determined) there is practically no localized protoplasm present. Its entrance presumably starts the transformation of yolk into protoplasm. During the passage of the sperm cell to the centre of the egg the protoplasm gradually accumulates, until on its arrival there it is surrounded by a conspicuous amount. Here it remains stationary until the egg nucleus, having been transformed in its peripheral position into the female pronucleus, returns to the centre surrounded by a small mass of protoplasm, the two nuclei eventually uniting to form the cleavage nucleus.

Many of the unsegmented eggs apparently undergo a process of degeneration, the process consisting chiefly in the formation of a large number of oil globules, which are protruded between the surface of the egg and the membrane; these form large masses on the surface of the egg. It is probable that such eggs failed in fertilization, and are consequently degenerating. Sections show that the yolk material is undergoing pathological changes and becoming clear in spots. The external membrane adheres more closely to the surface than in the case of the normal egg. There is, moreover, no vitelline membrane present. On a large number of normal eggs, so large a number as to suggest its constant presence, is a small raised spot, conspicuous enough to be seen on the whole egg. In section this appears as an irregular protoplasmic mass with apparently no definite structure. It possibly represents the place of entrance of the sperm cell; for a time it remains visible, but is eventually absorbed or otherwise disappears. The vitelline membrane offers no particular points of interest; it is very thin and reacts strongly to a protoplasmic stain; its color is nearly the same as the protoplasm of the outer surface of the egg.



*Cleavage and Blastoderm Formation.*

The cleavage in Anurida, and as far as observed in the rest of the Collembola and Thysanura, forms a striking exception to the method typical of the other Insecta. In Anurida the spherical egg has a cleavage that is slightly unequal but distinctly holoblastic. The first cleavage plane cuts the ovum into two practically equal halves (Figs. 16, 17). The second planes appear at slightly different places in the two halves and result in the arrangement of blastomeres so frequently found in annelids (Figs. 18, 19). Fig. 20 gives a different view of the 4-celled stage from that shown in Fig. 19, and illustrates the slightly unequal cleavage; this is almost a polar view and shows the shifting of the blastomeres. The second planes do not exactly halve the two parts of the egg. From this time on the planes appear regularly, the third being horizontal and the fourth more or less irregular, but in effect vertical, resulting in the 16-celled stage (Fig. 22). From this point on the details become confused by the rapid division and the difficulty of orientation. Holoblastic cleavage continues, however, up to the stage shown in Fig. 24, where a coarse morula stage has been reached. The cleavage planes are still distinct and the different cells stand up distinctly on the surface. After this, however, a change takes place that is clearly visible on the surface; the cell outlines become indistinct and the blastomeres flattened; until after another division the surface appears almost uniformly thickly scattered with white spots that represent the nuclei and surrounding protoplasm.

A study of sections shows what has been taking place. Beginning with an egg slightly younger than that one shown in Fig. 16 the first cleavage spindle is distinctly seen (Fig. 28). The spindle does not differ in any way from the usual type. The centrospheres are represented by the darker haloes surrounding the ends of the spindle, and scattered through the protoplasm are small yolk granules in the process of transformation into cytoplasm. Fig. 29 shows the reorganization of the nucleus and the amoeboid processes of the migrating masses of protoplasm. The number of chromosomes has not

been definitely determined, owing to their extremely small size, but the indications are that there are eight in each nucleus. Fig. 31 is a section through an 8-celled egg at the line  $z-z$  in Fig. 21. It shows the distinct holoblastic cleavage and four of the cells. There is practically no central cavity, the cells being crowded in together. The nuclei and protoplasmic masses are already preparing for the 16-celled stage; as this division is in vertical planes, the nuclei divide in a horizontal plane and can be seen in this section. In Fig. 32, the 32-celled stage represented in Fig. 23 has been cut horizontally at about the level of the line  $y'-y'$ . This shows distinctly that the typical blastula does not result from the cleavage; some of the cells have been crowded into the interior so that a solid morula results. This condition is still more evident in sections of the typical morula stage shown in Fig. 24. Up to this point the cleavage has been undoubtedly holoblastic and practically equal, for the slight inequality shown in the 4-celled stage becomes more and more obliterated by subsequent divisions. After the morula has been formed a decided change takes place in the internal structure corresponding to the external features already described. In Fig. 33 there is shown a gradual obliteration of the hitherto distinct blastomeric outlines. The nuclei and protoplasm of the outer blastomeres have migrated to the surface, leaving the yolk masses on the inside. In the inner part the nuclei and surrounding protoplasm have entirely left the yolk masses and are evidently moving towards the surface. There has been a cessation of the total cleavage, and now the blastoderm is being formed by the migration of the cells from the blastomeres to the exterior. Consequent on this change the yolk is left behind as an inert mass; and, though at first retaining a separation into masses corresponding to the earlier blastomeres, it gradually assumes a more compact arrangement. The cells, if this name can be applied to the migrating masses of protoplasm containing a nucleus, divide as they pass to the exterior, and some remain behind, also undergoing the process of division. Fig. 34 shows the final result of this migration. Here a blastoderm has been formed by migration

from the holoblastic egg. The cells have arranged themselves in two definite layers which differ slightly in character. The outer layer is continuous, while the inner is composed of fewer cells at regular and greater distances apart. Some cells remain behind in the yolk. Following this there is a rapid division of the cells forming these two layers, until a stage shown in Fig. 35 is reached. Cell outlines become very indistinct in the blastoderm and the size very much reduced. The protoplasm becomes strongly vesicular. Some of the cells left behind in the yolk cease division at a much earlier stage and remain large, lying in the yolk; others are grouped in masses (Fig. 35, *en.*).

These are the principal steps in the cleavage and formation of the blastoderm as found in Anurida, and they can be seen to widely diverge from the centrolecithal cleavage and consequent migration of the cells typically found among insects. Unequal holoblastic cleavage has been described by Lemoine ('87) in *Smynthurus* and retarded holoblastic in *Anurophorus*. Ryder ('86) while describing some of the later embryological features of Anurida does not consider the early stages, so makes no note of the cleavage. *Smynthurus* and *Anurophorus* were not studied in section, so that internal changes were not described. In Anurida, as has been seen, there is a sudden change in the method of development, which results in the final formation of the blastoderm by migration, as in the case of the typical centrolecithal egg. The temporary preservation of the yolk blastomeres suggests the condition found in a centrolecithal egg after the blastoderm is formed and secondary yolk cleavage has occurred.

Before discussing possible interpretations of the facts observed in Anurida, a brief sketch of some other somewhat peculiar methods of cleavage will be attempted. There is no other arthropod as yet described in which cleavage takes place in just this way. Many are holoblastic at first and change their method of cleavage during development. Among the crustaceans are found some interesting forms. Korschelt and Heider ('92) classify crustaceans according to cleavage methods, and put in the second group all those forms which start with

holoblastic cleavage and eventually lose it. Brauer's ('92) figures are given for *Branchipus*, in which the change takes place in the following way: holoblastic cleavage results in a distinct and regular cleavage cavity. This method continues until a layer of small, narrow, and very long columnar cells is formed, having their nuclei on the periphery and long yolk stems extending in to the centre. Eventually, this inner part forms a fused yolk mass with a single layer of blastoderm cells on the outside not separated from the yolk. *Alpheus*, *Palaeomonetes*, and *Hippa* all have a similar cleavage according to Herrick. Ishikawa ('85) states that in a fresh-water form some cells remain behind; these are, he thinks, doubtful in significance. Among the pantopods there are two methods of cleavage. Morgan ('91) describes the cleavage in *Pallene* as at first total; gradually the nuclei become more and more peripheral, and eventually the cleavage planes in the yolk mass are lost. *Tanystilum* and *Phoxichilidium* present another variation; holoblastic cleavage is maintained up to the 16-celled stage with the formation of a central cavity (Figs. IX, X). This is followed by a delamination of the entoderm from the inner ends of the single layer of cells (Fig. X, *A*, *B*), filling the blastocoele with entoderm cells. In this way entoderm arises by what might be considered a process of multipolar migration.

Among the myriapods certain phases are suggestive. Zo-graff ('90) figures *Geophilus* as having purely centrolecithal cleavage of the nucleus, accompanied by a certain kind of yolk cleavage (Fig. XI, *A*, *B*). This gives the outward appearance of holoblastic cleavage that has been claimed as general throughout the group. The central nuclei now migrate outwards along these yolk cleavage lines, and the blastoderm is formed by migration. Small masses of cells are found at the ends of each cleavage line that eventually spread out and form a complete blastoderm (Fig. XI, *D*).

It is clear from these few cases that change in method of cleavage is a widespread fact among arthropods, but accomplished in many different ways. *Anurida* differs from any of the others described. As in *Branchipus* no blastocoele is formed, the strictly one-layered condition is early lost, and a



condition suggesting Fig. X, *B*, is reached (compare Fig. 32 and Pl. XXII, Fig. X, *B*). Later the relations are more like those in Fig. XI, *C*. This suggests a line of possible explanation for the conditions found in *Anurida*. These cells found inside the morula, as shown in Fig. 32, *c*, *b*, may originate by a multipolar immigration, which is the primitive method of invagina-

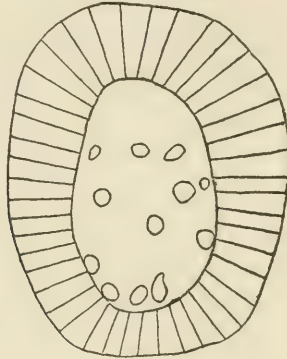
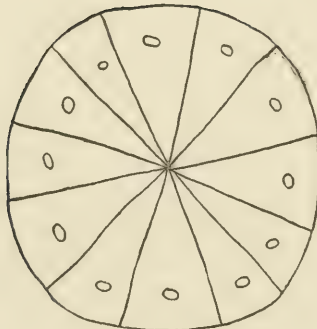
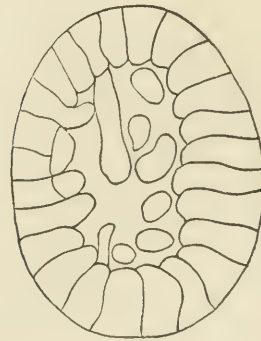


FIG. IX.

FIG. X, *A*.FIG. X, *B*.

tion. As the second layer of the blastoderm is formed largely by the migration of cells that were earlier inside the morula, it may be considered to arise by an imperfect and incomplete gastrulation. Whether the layer thus formed by migration from the inner part of the morula receives any additions from the outer layer is uncertain. Cells in various stages of division with the spindle axes directed obliquely to the surface point to such a possibility. The origin of the many cells re-



maining in the yolk is undoubtedly from those cells that were inside the morula. As to the names to be applied to these determinate layers of the blastoderm there is no doubt. Without hesitation the outer can be called ectoderm and the second



FIG. XI, A.

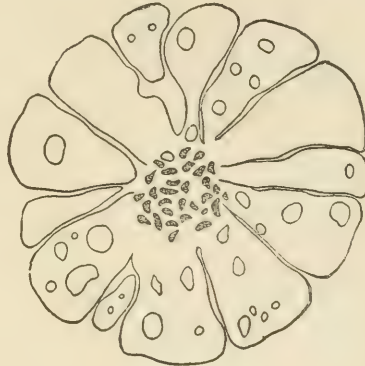


FIG. XI, B.

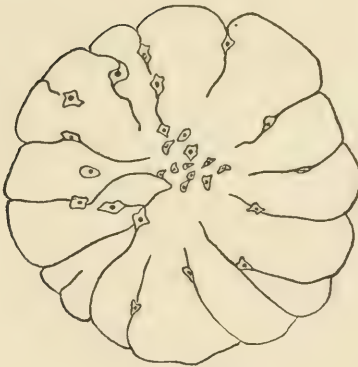


FIG. XI, C.

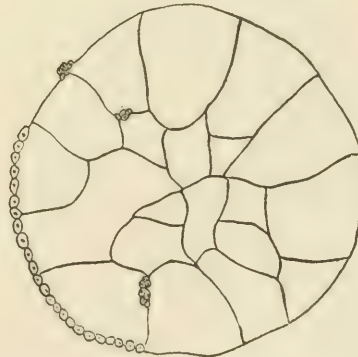


FIG. XI, D.

mesoderm, while, as will be shown later, the entoderm develops from certain of the cells left in the yolk. It remains dormant, however, until a late stage in embryonic life.

#### *Precephalic Organ and Blastodermic Membranes.*

For a certain time after the blastoderm is fully formed further changes consist chiefly in increase in the number of cells and decrease in their size, the lower layer always main-

taining the numerically established ratio of cells (Fig. 35). The next change is that in a certain place the blastoderm cells cease to divide, and assume certain definite characters. This is the beginning of the "precephalic organ," or, as it has been called, the "dorsal organ." Figs. 35-39 illustrate the stages by which the organ attains its full size. After the nuclei cease to divide, the cytoplasm begins to increase in amount and becomes highly vesicular in structure, forming a thick layer (Fig. 35). The nuclei increase in size, but do not divide either kinetically or akinetically. These changes apply only to the ectodermic cells. The mesoderm cells lying below these disappear gradually, partly by migration and partly by disintegration. At first the protoplasm accumulates more rapidly below the ectodermic nuclei, placing these on the periphery, but soon a process of infolding and insinking begins. The nuclei gradually sink lower as the amount of protoplasm increases, until a condition shown in Fig. 37 is reached; this is quickly followed by the stage of greatest development shown in Fig. 38. Here the precephalic organ (*pc.o.*) resembles a large gland; on a whole egg it appears as a large, circular, lighter mass that is clearly of some depth. By dissecting it out, the organ is found to have the form of an oblate spheroid, as would be inferred from its form in sections. During the development of the organ by a process of invagination, the surface over which it reaches is much reduced (Figs. 36-38), but the number of cells remains the same. The necessary crowding down of the nuclei in this process causes them to remain at different levels, suggesting that the organ is composed of several layers. This is, however, simply an appearance, there being only one layer of cells and these ectodermic. The cells always remain distinctly separated from each other, and the protoplasm is very vesicular.

The next change is a striking one; the vesicular character of the protoplasm is supplanted by a strongly marked striation that appears first at the outer edge in vertical planes. The final result of this process is seen in Fig. 39, where the nuclei are crowded to the bottom of the organ, which is irregular in shape and apparently beginning to degenerate. The outer ends of the cells have been elongated and drawn out into fine threads,

which, after being constricted to a rather narrow neck on the outer surface of the blastoderm, spread out like a fan or, when seen in the uncut egg, like a mushroom. In order that the later stages of this structure may be clear, it is necessary to consider some changes that have taken place in the blastoderm in general. The protoplasm of all the ectoderm cells has been increasing in amount and becoming vesicular, forming a deep protoplasmic layer (Fig. 37, *e.c.*) over the surface. This preparatory stage is followed by a rearrangement of the ectodermic nuclei, so as to form a wavy line (Fig. 38, *e.c.*); the protoplasm also assumes this form. No cell outlines are distinguishable; the whole blastoderm appears a continuous sheet of protoplasm, containing nuclei at certain definite intervals. After a short time, a very thin membrane separates from the surface. This is formed of thin strands connecting thickened masses (Fig. 40, Pl. XXII, *p.*). The latter are found to have come out of the troughs of the folds in the ectoderm, the thin strands from the crests. A thin layer is formed on the precephalic organ. Soon after, a layer of protoplasm is separated from the blastoderm having a definitely crenated form (Figs. 38, 40, *c.1*, Pl. XXII). The last part of the process is again repeated, and a second crenated membrane is formed (Fig. 39, *c.2*). In the region of the precephalic organ, this last membrane has peculiar relations. The elongated ends of the cells spoken of earlier are found to be directly connected with the second crenated membrane by thickened ends, which appear as a knob in cross-sections (Fig. 39, *k.*). These envelopes are developed in a way closely similar to that by which the original egg membrane is formed. The superficial protoplasm is at first markedly vesicular, and, after becoming homogeneous, is separated as a uniform layer. When the second crenated membrane is nearly formed, the ectodermic nuclei return to their original plane and the two layers again become parallel.

At the end of this process there are, surrounding the egg, five membranes. First, the egg membrane formed in the ovary but by the egg (Pl. XXII, Fig. 40, *e.m.*); then the vitelline membrane, a thin but distinct envelope (Pl. XXII, Fig. 40, *v.*). This is followed by *p.*, the material cast off preparatory to the more

complex structures following; it is very thin excepting in places where the knobs are attached, which came from the trough of the fold. The first crenated membrane (*c.1*) is the next. This is uniform in thickness and is shed over the whole surface, including the precephalic organ, which is, however, unaffected by the crenations. By the time the last envelope, the second crenated membrane, is formed (Fig. 39, *c.2*), the dorsal organ is undergoing the process of degeneration already described; its cells are elongating, the inner edge is becoming uneven, and the nuclei are shrinking to more solid masses (Fig. 39, *pc.o.*). The close connection between this organ and the envelope is readily proved by the fact that when the membrane is removed the organ is usually torn away from the embryo and remains attached to the envelope. No suggestion as to the function of the precephalic organ can be made; there was no evidence that it is particularly associated with yolk absorption; its period of greatest development precedes the appearance of the germ band. But its only obvious use begins at about this time. The ultimate fate of the structure is gradual absorption; it becomes smaller and smaller, as is seen at *pc.o.* in Pl. XXIII, Figs. 41-45. It loses its connection with the envelope, and remains recognizable as a darker red staining mass inside the embryo, with a tuft of fine threads outside. On the hatching of the animal it is no longer visible.

From these facts it is clear that there is no structure present corresponding in origin and nature to the amnion and serosa of the other Insecta. These are distinctly cellular envelopes, and appear at a later time. The "dorsal organ," so-called among the higher Insecta, is directly connected with these envelopes. Lemoine ('87) has discussed this organ among the Poduridae, describing its form in *Anurophorus* and *Smynthurus*; he also suggests its probable relations to the structures found in the other Insecta. The organ forms a conspicuous part of the embryo in both these genera; in the former it appears early in the development of the blastoderm, but in *Smynthurus* its appearance is delayed until the formation of the ventral plate, and it persists until hatching. As no sections were cut, the discussion of the relations existing between this organ and the



envelopes and their probable connection with similar structures in other hexapods is somewhat vague and unsatisfactory. One point is clear, however: there are membranes in both forms that are attached to the organ, and constitute a structure important to the embryo. Lemoine says there are present at first in *Anurophorus* an outer thick "chorion," which is perforated irregularly in numerous places, and an inner very fine vitelline membrane. Later, more membranes are formed, the first of which appears after the formation of the blastoderm; it is described as very fine and not uniform in thickness. The author judges it to be formed of many cells identical in origin with the blastoderm cells, and calls it the amniotic membrane, naming it, however, purely from analogy of form and function. This "amniotic membrane" is connected with the dorsal organ by an ampulla, which Lemoine calls the "amniotic ampulla." Throughout the greater part of development another membrane is also present that he considers a true larval skin, as it forms on the outer parts of the appendages also.

Nicolet describes two envelopes for the poduran embryos he studied (*Podura*, *Cyphodeirus*, *Desoria*, *Smynthurus*), the outer very stiff and the inner fine, probably corresponding to the "chorion" and vitelline membrane of Lemoine. Oulganine ('75) describes two similar structures in *Achorutes*, *Anurophorus*, and *Degeeria*. It is evident that these two membranes are found in all these forms, but Lemoine is the only one to describe still more. Leaving out the two preblastodermic envelopes that are similarly described by all authors, one of the inner ones described in *Anurophorus* and *Smynthurus* may be considered as resembling the crenated membranes described in *Anurida*. This "amniotic membrane" of Lemoine is peculiar in its behavior during the life of the embryo. It possesses great powers of expansion and contraction, increasing the size of the egg by one-fourth or one-third at its largest size compared with its smallest. The author goes further and states that in contraction a folding of the surface of the embryo takes place, giving it a roughly four-sided figure with fine wrinkles over the surface.

No such powers of rapid contraction were seen in *Anurida*.



In living specimens the space between the embryo and the membrane is not very large, but it is practically constant; in the early stages the crenations are narrow and deep (Fig. 38, *c.1*), but later they are wider and shallower (Fig. 39, *c.2*). This is clearly associated with an increase in the size of the embryo, as measurements prove. Very soon after the formation of the membranes the first, the egg membrane, splits, and with it the vitelline membrane. Then the first crenated membrane becomes the outside cover, and a decided increase in size is observable. Growth continues until eventually the wrinkles are expanded so as to make the crenations flat in comparison with earlier stages. The attachment of this inner crenated membrane with the dorsal organ serves as a means of suspension of the embryo in the envelopes; it is thus held in a fixed position. In preserved specimens, in which some amount of shrinkage of the embryo has taken place, the space round the embryo is considerable. The latter hangs eccentrically placed, owing to its attachment to the membrane. There is one other possible use for the crenations in the envelope besides the simple one of allowance for growth. The eggs are subject to considerable variations in pressure and degrees of moisture, owing to the changes in level of the tide. The crenated surface would more readily resist the effects of this change in pressure than an unfolded one. Observations were attempted to determine this point, but nothing definite resulted, and any such suggestion must remain an inference.

It seems clear that powers of expansion belong to the embryonic envelopes of at least three of the poduran genera,—*Smynthurus*, *Anurophorus*, and *Anurida*. As regards the causes of such changes, there is less known; in *Anurida*, growth and possibly changes of pressure are the direct agents, while in *Anurophorus* and *Smynthurus* contraction and expansion take place regularly without any apparent external or internal cause. Lemoine's Fig. 16 strongly suggests another interpretation of the crenations found in the embryo; it so much resembles the early crenated stages of *Anurida* as to make it possibly a corresponding stage, instead of an embryo undergoing excessive contraction.

Leaving the podurans, there are points of interest to be found in connection with the higher groups. Wheeler ('93) discusses a curious structure found in *Xiphidium*, which he calls the "indusium." It appears at the same time as the germ band, or a little later, and is ventrally placed on the long oval egg, just in front of the head. It remains unchanged for some time, usually separated from the head, but sometimes connected by a small string of cells. Ultimately by proliferation it forms an envelope, pushing its way between the serosa and the yolk, and finally becomes an inner membrane next to the yolk, and only separated from the embryo by the amnion. Strange to say, this organ forms for itself an amnion, which spreads round the egg and is recognized as the outer "indusium." The author homologizes this with the poduran "micropyle," and seconds the previous suggestion that the latter is truly homologous with the "dorsal organ," as found in some groups of the Crustacea.

Among the Crustacea there are found curious intermediate structures. Bobretsky ('74), in his studies of *Oniscus*, which have been corroborated in part by Nusbaum ('86), discusses the so-called "primitive cumulus" or "dorsal organ." Its origin as described is similar to that of the precephalic organ in *Anurida* both in manner and in time. Excepting in the dorsal half of the embryo the two germ layers are distinct. After remaining stationary for a long time the cells increase in number and spread out over the dorsal part of the embryo as a cap. This cap is connected with a thin membrane that the author calls a larval skin. At the greatest development of this organ it remains as a saddle-shaped cloak composed of a single layer of cells.

In embryos of two species of *Idotea* found at Woods Holl, a structure similar in its early stages to that of *Anurida* was found, but it was paired, one small organ being placed on each side of the middle line. This resembled closely Nusbaum's ('87) figures of *Mysis*.

To make a graded series between *Anurida*, *Oniscus*, and *Xiphidium* is easy. In the first the organ is large, active, and functional in very early stages; it later begins to degenerate and assumes certain secondary characters, as, for example, the

connection with the membranes. In *Oniscus* the cells migrate bodily instead of simply elongating, and form a cellular cap instead of a membranous one. In *Xiphidium* the cellular envelope is completed and entirely encloses the embryo. Wheeler's suggestion is that the organ he calls the "indusium" had probably lost its original function, and was degenerating and varying in consequence; accidentally acquiring a new value, it was reconstructed for its new use as an embryonic envelope. In *Oniscus* this process of reconstruction is not yet completed, and in *Anurida* barely begun. There is an interesting suggestion in Wheeler's ('93) mention of the embryonic sucking disc in *Clepsine*. A complete series may possibly be made between this disc, the organ as found in *Anurida* and the phyllopod cervical gland which actually functions as a sucker, and is regarded by Müller ('64) and Grobben ('79) as the homologue of the "dorsal organ" of the Amphipoda. In this case the power of adhesion that still belongs to the precephalic organ in *Anurida* is possibly a remnant of its former function. The gradual prolongation of embryonic life causes the young to hatch in a more mature stage, and need for the adhesive disc of the immature larva is lost.

#### *Embryo Formation.*

After the separation of the second membrane the formation of the ventral plate or germ band begins. On surface views it first appears as a narrow band passing round the egg in such a way that it nearly encircles it, the precephalic organ being the separating mass. The head of the embryo lies on one side of it, and by crossing the organ the tail is found at the opposite side (Pl. XXIII, Fig. 41). Almost immediately the outlines of the embryo can be distinguished, the different parts being laid down successively from the head backwards. The mesodermic somites indicating the future segments of the body appear, and almost at once the appendages of the different parts. In as early a stage as that shown in Pl. XXIII, Fig. 41, the beginnings of the antennae, mandibles, maxillae, and thoracic legs are evident, and Pl. XXIII, Fig. 40, shows an added pair of

appendages between the antennae and the mandibles, as well as the faint outline of the rest of the germ band. Up to the stage figured in Fig. 42 the chief changes are in the clearer definition of the six abdominal segments, the appearance of the median unpaired labrum, and the indication of the proctodeal and stomodeal invaginations. The antennae have become undoubtedly three-jointed, with an indication of a fourth, and the precephalic organ has begun its process of elongation and degeneration (Fig. 42). The embryo still preserves its spherical form, and stretching across between the ends of the appendages can be seen the last envelope formed (Figs. 42, 44, *m.*). Whether this is a true larval skin or is similar to the "Blastodermhäuten" already discussed is not clear, but it can best be seen after the appendages have appeared. It seems most likely that it is shed just at the beginning of the embryo formation; it passes round the embryo, and is frequently found attached to the ends of the precephalic organ.

From this point a radical change of form takes place; a flexure of the embryo begins that results in crowding the mouth-parts together to form a definite head and folding the embryo upon itself. This greatly changes the points of reference in regard to the precephalic organ: the head remains in about the same position, but the tail is drawn much farther away, and the embryo becomes restricted to less than one-half of the circumference of the egg, instead of extending over nearly the whole. At the same time there is a marked lateral flattening, so that the young animal is much narrower measured from side to side than measured dorso-ventrally. Before the final stage of this process is reached, however, certain features of note have appeared. The most striking of these are shown in Figs. 43 and 45. Fig. 43 represents an embryo in which the ventral flexure has just begun, as shown in the side view of Fig. 43, *a*. The brain lobes, the protocerebrum, have clearly appeared, and their elongation into optic lobes is evident. The labrum, unpaired, and lying on the middle line, is seen just anterior to the stomodaeum. The antennae lie on each side of the future mouth, and are formed of three short, thick, and approximately equal joints. The three pairs of



mouth-parts are next in succession. On each side of these has appeared a ridge that passes backward along the embryo, the two folds enclosing the mandibles and maxillae. These folds start from just the region where the small intercalary appendages were seen earlier, but which have now disappeared. Figs. 43, 46, and 47 show the process by which this change takes place, and leave no doubt that the folds as they finally appear are a development from the intercalary appendages. This sheath-like form of the extra mouth-part explains the well-known peculiar structure of the adult head. The adult mouth has always been described as deeply sunk into the head and appearing as a tube, out of the end of which the points of the mandibles and maxillae protrude. It can be readily seen that the labrum in front and these lateral folds make together a three-sided box in which the mouth-parts, two mandibles, and four maxillae are sheltered.

In Fig. 43, where flexure has just begun, the thoracic appendages are visibly longer and more distinctively legs. The first abdominal segment bears a large pair of appendages that are ultimately modified to form the collophore, while on three of the succeeding abdominal segments there are also small appendages, those on the fourth segment being the largest. This condition is equally evident in Fig. 44, a slightly later stage. In Fig. 45 the conditions are still the same; the collophore is, however, almost hidden by the flexure of the body, and the terminal segment has elongated into two decided folds that surround the proctodaeum. The five single eye-spots have appeared on the sides of the head, and the precephalic organ is much reduced and shows the thread-like elongation of its cells.

An interesting question is raised by a consideration of the folds that rise round the mouth. The simple structure of the adult mouth in these forms has been discussed by Fernald ('90); he describes it as being a pouch of considerable size, at the inner end of which are attached two pairs of jaws; these are entirely enclosed. He cannot, however, determine the exact homologies of the different parts. Hansen ('93) discusses the homologies of the mouth-parts of the Crustacea and Insecta by studies on *Japyx*, *Campodea*, and some of the *Collembola*. He finds it a



common peculiarity that the mandibles and maxillae are sunk deeply in the head up to the points, as in the case of *Anurida*. He speaks of a fold of the skin that causes this insinking which is attached to the labrum, and is undoubtedly similar to the fold found in *Anurida*. *Campodea*, *Japyx*, *Machilis*, and *Lepisma* all agree in general details, but the last mentioned is considered by Hansen a transition form between the *Thysanura* and the *Orthoptera*. The relations as seen in the anuridan embryo are as follows: The unpaired labrum forms the upper part, the front of the pouch, at the back of which work the two pairs of jaws, the mandibles, and the first maxillae, while the second pair of maxillae has been modified to form the back of this pouch. The lateral folds already described make the sides and are developed as shown from the intercalary segment.

The question naturally arises as to what homology this additional pair of mouth-parts can have, arising as it does on a distinct segment. Viallanes ('91) and Wheeler ('93) agree in the following structure of the orthopteran head and brain: It consists of a protocerebrum, the most anterior segment, forming the mass of the supra-oesophageal ganglion, from which the large optic nerves are developed. A deutocerebrum and tritocerebrum follow, which together complete the brain and the oesophageal collar. Following this is a series of ganglia corresponding to the mouth-parts, which eventually fuse to form the suboesophageal ganglion of the adult. These authors find distinct mesoblastic somites in the segments of both the deutocerebral lobes, and hence conclude their equivalence in value to any of the succeeding segments. The antennae of insects are enervated from the deutocerebrum, and, as has been demonstrated by Viallanes ('91) and St. Remy ('90), the first pair of crustacean antennae is also connected with this brain lobe, the second pair being enervated by the tritocerebrum. Hansen homologizes the mandibles in the two groups, but does not decide on the antennal homology. It would seem clear from the work already mentioned on the brain that the homology of the first antennae of the Crustacea with that of insects is practically decided. Arguments drawn from the absence or presence of either pair of antennae in the higher

Crustacea are not convincing, as there is great variation in the degree of development of these appendages in different groups. In some the first antennae are larger and the second small or absent, and in others the reverse is true. The evidence from the lower forms is more reliable. Ray Lankester enumerates the different appendages found in that archaic type *Apus*, and indicates that the first antennae are always present while the second are sometimes absent and sometimes present, in the same species, and always missing in some species. As *Apus* is considered more generalized in its structure than any other crustacean, it is suggestive that the first antennae should be constant and the second the more variable. This immediately suggests an interesting explanation for the added pair of mouth-parts found in *Anurida*, originating from the tritocerebral segment. On this basis they are a modified form of the second pair of antennae in the crustacean; and hence *Anurida*, including, without doubt, its allied forms, possesses an adult structure clearly homologous with the second antennae, the very important appendages of some crustacean heads. It is interesting in this connection that Hansen considers the musculature of the head of *Machilis* much more like that of the crustacean than that of the insect.

Fig. 48 represents an *Anurida* just hatched. It can be seen to have many of the characters of the adult form; it is, however, perfectly white, showing none of the black pigment characteristic of the adult. The surface of its body is not as wrinkled and folded at this young stage, and the cuticle lacks the finely papillose surface found in the older specimens. The antennae are clearly four-jointed, as described by Ryder ('86); the terminal joint is less pronounced, however, and sometimes is not completely separated from the third. Instead of a third joint there exists only a constriction. The collophore is prominent; it originates by the fusion of the two appendages on the first abdominal segment. Young animals at this stage are very active, and may be found in large numbers in the same places as the eggs. Judging from the great variation in the size of the eggs at the time of hatching, there is a great variation in the size of the animal; this must be the case,

because it is not a rule that the smallest is the least developed. Quite often the smallest ones have undergone considerable post-embryonic development, while some larger ones are much farther back in the process. Pigmentation and increase in size are the chief external changes that are needed to make the young *Anurida* resemble the adult. Both of these characters come slowly, though the young are probably all pigmented by the end of the season. They remain small in size, however.

Comparing these results with Ryder's ('86) figures, which are, as far as known, the only published studies of the embryonic stages of *Anurida*, certain differences are observable. There are figured in these the two crenated membranes and the early stages of the germ band, several later embryonic forms, and young and adult animals. The chief difference in the embryos as figured by Ryder and those shown in Pl. XXIII of this investigation lies in the different interpretation of the embryonic head appendages. Ryder recognized but three pairs: one pair of antennae, one pair of mandibles, and one pair of maxillae. He included the second maxillae with the thoracic legs, and did not see the intercalary appendages. In the recently hatched young he describes a structure placed on the anterior part of the fourth abdominal segment which he considers represents a rudimentary spring. No evidence of such a structure was seen in the young investigated, and large numbers were examined. In the embryonic stages the appendages on the fourth abdominal segment are larger than any of the others, excepting those on the first (Fig. 45, *a.4*); but these, like all the others excepting those on the first, disappear before hatching.

The process by which the germ band arises is exceedingly simple. Immediately after the formation of the second crenated membrane, or even before, or in some cases before all the entodermic nuclei have sunk to a common level again, the mesoderm cells may be seen migrating to such a position that one meridian passing through the precephalic organ and the centre of the egg would cut the band they form longitudinally into two. The migration eventually leaves the greater part of the egg covered only by ectoderm and the germ band appears



girdling the egg. At first one-layered, the mesoderm early shows a further change to two in certain parts which represent the mesoblastic somites. Fig. 49 represents a cross-section through the germ band just after its formation. In the middle line, under the median ectodermic depression, the mesoderm is a single layer of cells; but on each side there are the early indications of the somites. The two-layered condition arises by migration, and the cavity when present is hollowed out afterwards. Subsequent modifications arise by differentiation from this primitive condition.

*Origin and Development of the Entoderm.*

The place and manner of entoderm formation long remained in doubt, as the appearance of the mid-gut is delayed till very late in embryonic life, and these late stages are difficult to find. However, the following facts and explanation were eventually determined. By the end of cleavage two definite layers are fully established, the ectoderm and mesoderm, as shown in Fig. 35. There are, moreover, certain cells left in the yolk that have never taken part in the formation of either of the two layers. Some of these that are spread singly through the yolk are evidently yolk cells, and function in the transformation of yolk for the nutrition of the embryo (Figs. 35, 37, 39, *y.c.*). In addition to these, however, there are some cells that remain grouped in clusters, the whole mass evidently arising by division from a single cell or perhaps a few cells. The clusters are placed above the centre of the egg, using the precephalic organ as the pole of the reference axis, and very frequently limited in numbers to two (Fig. 35, *en.*). One of two things now happens to them: they either migrate from the masses and are scattered through the yolk in small groups of twos or threes, or else they remain unchanged for a considerable length of time. In the former case they are difficult to distinguish from the yolk cells, but their greater transparency, larger vesicular cell bodies, and association in small groups is a sure guide to their identification (Pl. XXII, Fig. 41). The yolk cells early acquire a more deeply staining nucleus, showing the characteristic increase of chro-

matin in cells with a strongly assimilative function. In the second case, when these cells remain permanently associated in one or more clusters, no further change occurs until late in embryonic life. By the subsequent development of the embryo and its changes in form during flexure, the relative positions of the groups are somewhat changed. One large mass may, however, readily be recognized in the region of the proctodaeum, not far from the ectodermal layer.

After the embryo has reached a stage corresponding to Fig. 45, or perhaps later, the mass may be seen to be scattering, and certain changes occur in the yolk. Around some cells are large spherical masses of yolk particles contained in extremely vesicular protoplasm, in which there is a small central nucleus (Fig. 58, *en.*). These are particularly numerous in the regions of the stomodaeum and proctodaeum, and may be clearly seen later to assume a regular arrangement in the yolk, forming two broken lines reaching through the body. It is now clear what these mysterious cells are: they are the entoderm, and are taking up definite positions to form the mesenteron of the young animal. In a newly hatched specimen an interesting relation is shown; Fig. 65 represents part of a frontal section through the body of such an animal. The mesenteron is seen to be composed of large irregular-shaped cells with extremely vesicular protoplasm; the nuclei are irregular in size and stain faintly. At the inner or free edges of the cells masses of yolk are visible, and certain of the cells also contain similar particles. Whether these particles are passing out of the entoderm cells to the enteric cavity or are being ingulfed by them is not clear, but in either case it is evident that the mesenteron when fully formed contains very little food yolk, a condition contrary to the general rule. The entodermic cells are resting on an extremely thin membrane, but there is as yet no sign of muscular walls or other differentiation. The cells are themselves still irregular and almost amoeboid in form (Fig. 65, *en.*).

This is, then, the history of the entoderm in *Anurida*; it originates without doubt during cleavage, and takes up its position in the middle of the morula by a process which it is possible to call invagination. When the mesoderm, which also lies within



the morula, migrates outward and forms a definite layer below the ectoderm, the entoderm remains in the interior as one or more cell masses and is comparatively unchanged till a late period of embryonic development. Finally the cells separate and increase in size, and ingulging yolk arrange themselves to form the definitive mesenteron, which contains practically no yolk excepting some in an intracellular condition. Whether the vitellophags are genetically connected with entoderm is not clear, but they very possibly are entoderm cells that early assume their digestive powers. That they do not, however, take part in the formation of the mesenteron is clear from their presence at the time of its formation scattered throughout the yolk, recognizable as shrunken degenerating bodies (Fig. 57, *y.c.*).

At first sight this process is markedly different from that described for other insects; but the differences admit of quite ready explanation. The typical process of entoderm formation in the other Insecta is by proliferation from two formative centres, an oral and anal, that appear at the two ends of an elongate blastopore. This process has been demonstrated for the Coleoptera by Heider ('89) and Wheeler ('89); in the Diptera by Voeltzkow ('89) and Graber ('89); in the Hymenoptera by Carrière ('90); and in the Orthoptera by Wheeler ('93). In several other forms but a single formative centre is described, that one being the anal. From this one or these two centres a continuous band is formed by proliferation that finally incloses the yolk completely. The great difference between the two processes, as described for Anurida and the rest of the Insecta, lies in the different disposition of the yolk; in the former case it is not inclosed in the mesenteron, and in the latter it is. The other variations may be harmonized in the following way: The groups of cells that are usually two in number can be considered to be the equivalent of the two masses in the higher Insecta; the difference in size is marked, the anal mass being the one more likely to persist in a recognizable condition. A similar difference was observed by Wheeler ('93) for *Xiphidium*, where he found the anal centre decidedly larger than the oral. The position of the two masses or, as it may be, one rather scattered mass just below the precephalic organ may be indica-

tive of its future anal position, although at this time the germ band is not yet laid down. By a somewhat early migration the entoderm cells are scattered, and finally assume their definite relations at a very late embryonic period. It is, of course, possible that the entoderm cells even at this stage assist in the transformation of the yolk, but there are certainly separate yolk cells for this duty.

As readily seen, the process of entoderm formation in Anurida agrees very closely with the method found among some of the Crustacea. In many members of this group the entoderm is early differentiated from the rest of the cells, but remains stationary for a long time, simply imbedded in the yolk. In some cases its origin is still under discussion; some authors claim that it is composed of vitellophag cells that have been functioning in the egg from the beginning; in many cases the entoderm only assumes its permanent relations at a late period of development. Zograff ('90), describing the origin of the mesenteric lining in two species of *Geophilus*, says that it appears from the yolk, and concludes that the process closely resembles that found in Malacostraca and Arthrostraca. Heathcote ('86) describes it for *Julus terrestris* in the following way: Certain cells during cleavage remain behind in the yolk and form the entoderm, and in turn give rise to the middle germ layer. After the appearance of the ectodermal parts of the alimentary tract the scattered entoderm cells arrange themselves to form a central lumen and give rise to the mesenteron. From these few points of comparison it is clear that Anurida constitutes an interesting intermediate form, connecting the processes typical of the crustaceans and myriapods with those of the higher Insecta. It is difficult to say to which of the lower arthropod groups Anurida is the more closely allied, especially as so little work has been done on the myriapods. Certainly the resemblances to the group last mentioned are very striking. The interpretation of the differences found between the higher insects and Anurida would point to a gradual delaying of the entoderm formation to a later embryonic period in those eggs possessing a larger quantity of yolk. This new relation possibly raises again the question as to whether all the cells resulting from cleavage of

the central nucleus in centrolecithal eggs pass to the surface to form the blastoderm. Some may remain behind and form the vitellophags and perhaps take other part in development. It is certain in Anurida that vitellophags do not migrate outwards and then return, but are left in the yolk.

*Development of the Reproductive Organs.*

The development of the germ cells was found to be one of the most interesting processes followed in detail. Their appearance takes place at a comparatively late period of embryonic life, the earliest stages occurring when the animal has reached the stage shown in Fig. 44.<sup>1</sup> At this period the processes represented in Figs. 50 and 51 are seen to take place in the second and third abdominal segments. Both these views represent cross-sections of the mesoblastic somites of one side of these abdominal segments, and show the great variation that occurs in the distinctness with which the cavities of the somites are developed. In Fig. 50 two germ cells (*g.c.*) are seen, one passing out into the yolk on the free side of the somite, and one as yet imbedded in the splanchnic layer of the mesoderm. These cells are readily recognized by their peculiarly clear transparent cell bodies. In Fig. 51 is shown another stage, when the germ cell is clearly inclosed in the cavity of the somite. Figs. 52, 53, and 58 show the line of development followed in this latter case. The germ cells are distinctly between the walls of the two mesoblastic layers, the splanchnic and the somatic. In Fig. 53 a definite form has already been attained by the germinal mass. There is a cephalic elongation and a hinder spherical mass. The surrounding mesoderm has been differentiated into muscles, and connective tissue is beginning to appear. There is, however, a distinct layer of mesoderm separating the germ cells from the yolk, the splanchnic layer; the germinal mass lying in a space appearing to be a true body cavity. In Fig. 58 the mass of cells is much larger, and by the crowding together of the abdominal segments it can be seen that flexure

<sup>1</sup> Since the reproductive organs are paired and the process is similar in general principles, descriptions will be made of but one side. The only difference lies in a slight variation in position of the two organs.

of the embryo has proceeded much farther. The continuous mesoderm sheet separating the germ cells from the yolk mass has been broken; at the most curved part of the mass of germinal cells there is direct communication between them and the yolk. Whether this breakage is due to rapid flexure or rapid increase in the number of germ cells, which show evidences of frequent division or to both causes, one thing is clear, that the germ cells are now in close contact with the yolk.

Returning now to a consideration of Fig. 50, the fate of germ cells originating in the second way may be seen in the series shown in Figs. 50 and 54-56. The single cell, set free on the outer side of the somite, increases to an irregular mass that lies in part sunk into the mesoderm and in part projecting out towards the yolk. These cells, at first a solid group, migrate outwards and begin to mingle with the yolk (Fig. 54, *y.*), the migration being most noticeable in the outer cells, those nearest the yolk. In Fig. 55 migration has not gone so far and the greater magnification shows the peculiarly "succulent" character of the cells. In Fig. 56, illustrating the extreme result of the process, the cells have divided into two groups, one (*s.g.c.*) remaining near the mesoderm and by repeated divisions increasing to a large mass of small cells, another (*m.g.c.*), which has migrated out and is spreading through the yolk, still maintaining, however, a certain relation to the stationary cells. Unfortunately, in spite of the most careful search, the latest embryonic forms found do not seem to supply the next step. The final result is seen in the figures in Pl. XXV, Fig. 64 concluding this series. This represents a longitudinal slightly oblique section of a just-hatched animal, showing the reproductive organs of one side of the body. At *g.e.* is a somewhat irregular mass of cells forming the germinal epithelium, lying in the second and third abdominal segments. Below this, and directly connected with it, is a large irregular sac filled with yellow material, in which are scattered a few large cells. Two lobes of this sac are cut through, and at its lower end, coming from the hinder end of the fifth abdominal segment, is an ectodermic invagination, the duct of the reproductive organs leading to the exterior. This animal is recognizably a young male, the parts described



corresponding to the parts of the adult. The yellow material in the sac-like parts of the organs is yolk (Fig. 64, *y.*), and the golden yellow globules scattered through the connective tissue is yolk acquiring the characters of fat globules. The whole body is very simple in structure, a few muscle fibres, the ventral nerve chain, and a large amount of connective tissue filling up the space being the essential elements; numerous blood corpuscles loaded with yolk present in the small body cavity were not represented in the figure. The reproductive organ is shown at a later stage of development in Fig. 65 in frontal section; the yolk has been absorbed from that part of the sacs near the germinal epithelium, and by rapid proliferation, probably rendered possible by the ample supply of food, very small sperm cells are being formed that will eventually mature as spermatozoa; these fill the sac.

In Figs. 59, 60, and 62 the story of the first group of germinal cells originating within the cavity of the somite is continued. Fig. 60 represents a cross-section of an ovary from a recently hatched or at least very young Anurida. The cut does not include the germinal epithelium, but some of the cells that have become detached from it are figured that show characters rendering their recognition easy. At *n.c.* are cells that bear all the distinctive marks of nutritive cells, large nuclei, richly supplied with chromatin, which is irregularly massed together, but not stellate in arrangement. At *o.* are seen cells that as distinctly possess the characters of ova, large cell body, and small clear nucleus. Scattered among these are granules of true embryonic yolk of irregular sizes. This is even included in some of the ova, as shown in Fig. 59. The ovarian wall is extremely thin; small nuclei occur at intervals that closely resemble the mesodermic nuclei of younger stages. Fig. 61 is a representation of an ovum with its nutritive cells, as found in an animal taken early in the summer, evidently before development had begun for the season. This shows how little, excepting in one respect, the ova and nutritive cells change during the winter. The one respect is in the chromatin of the nutritive cells; in the last figure the definite stellate structure is attained, while in the younger forms the chromatin



is in coarse threads (Fig. 62) or irregular masses (Figs. 59, 60). In the size and external characters of the animal the changes in the interval are marked, but practically no development has taken place in the reproductive cells. The external part of the reproductive organ, the outlet, was found to originate in the late embryonic stages by a median unpaired invagination from the hinder end of the fifth abdominal segment. As shown in the adult, the ectodermic part of these ducts is extremely short, being only the small unpaired part extending from the exterior through to the body space, where it joins the mesodermic part of the organ. This is found completely invaginated in late embryos, and showing in the female the accessory diverticula, the receptaculum seminalis, as a branch of the main duct.

When it was found that the yolk was not contained in the mid-gut of the embryo, the question naturally arose as to its final disposal. It has been seen from the above facts that a large part of it is included within the reproductive organs, and serves to hasten very much the maturing of the generative elements. A very large quantity is also found in the blood corpuscles of the newly hatched animal. Fig. 63 shows some of these taken from the same animal as Fig. 59. Gradually during their circulation through the body they must give up their rich supply of food. No complete observations were made on the origin of these cells, but it is probable that they arise from the mesoderm. Already in Fig. 53 small isolated mesodermic cells can be seen, and many are found later in different parts of the body. Even when loaded with yolk there is no possibility of confusing them with yolk-laden entoderm cells, they are so very much smaller in size (*cf.* Fig. 63 and Pl. XXII, Fig. 41; 63 is magnified more than 41). Yolk was found in the places already enumerated and also free in the body cavity, lying chiefly under the alimentary canal. Thickly scattered through the meshes of the connective tissue are many yolk spheres, which are eventually transformed into fat globules. When first hatched, the body cavity, as it is called, or, more correctly speaking, the haemocoel, is much obscured by a large amount of connective tissue that originates from the mesoderm. The alimentary canal, reproductive organs, and nervous system all

lie more or less imbedded in it, and it is only by later post-embryonic development that the space is finally cleared and becomes as distinct as in the adult.

The history and fate of the slight trace of the true coelom, as seen in the female embryo, has not been studied in detail and must remain a point for future investigation. As seen in Fig. 51, there is a distinct cavity in the mesodermic somite, although this is not so clearly marked in all the segments. In Fig. 58 the splanchnic layer of mesoderm forms one side of a spacious cavity, evidently resulting from the fusion of those parts of the somite cavities not cut off in the appendages. This space is a striking feature in animals of this sex and renders them immediately recognizable. It is, however, eventually obliterated; the beginning of this process is shown in the breaking of the splanchnic layer, thus allowing the germinal mass to leave the distinct true coelom. It is curious to find so much more reduction in the coelomic space in the male; it is as noticeably absent from the beginning as it was present in the female. At all times the germ band remains a solid mass; no space such as is shown in the female is ever seen.

The loss of the coelomic cavity is without doubt a derived condition, as the ancestors of the insects probably inherited the space more or less completely from their annelid-like progenitors. The retention by the female of primitive characters not found in the male is a frequent occurrence, the latter sex being the more subject to modification. The female is the more conservative and adheres more closely to the primitive type.

This inclusion of yolk in the reproductive organs is a point of great interest in the development of Anurida. It is a phenomenon not frequently observed in any forms, and, as far as known, without a direct parallel among the Insecta. There are certain forms, however, in which resemblances may be noted. Metschnikoff ('74), in his classic account of the embryology of *Polydesmus* and *Julus*, double-footed myriapods, speaks of the peculiar position of the nutritive yolk in the body of the young embryos. It is present almost exclusively in the body cavity; very little is found in the intestine. This is a fact true also of the daphnids, where the yolk lies in the body cavity between a

*yolkless* alimentary canal and the remaining viscera. Mordivilko ('95), discussing the structure and development of some of the aphides, speaks of Metschnikoff's "secondary yolk" (Metschnikoff, '66), and shows how it lies in the body cavity surrounding the reproductive organs and causing a wonderfully rapid growth on their part. Among the vertebrates certain forms exist in which a quantity of the embryonic yolk is associated with the germ cells, causing their rapid growth. Petromyzon, the lamprey, belongs in this category, and, as shown in Fig. 66, much of the yolk is included among the germ cells.<sup>1</sup>

*Summary.*

Summing up the results of this investigation on *Anurida* the following points are of interest:

(1) That the ovary is very simple in character, no arrangement corresponding to the ovariole of the higher hexapods being present.

(2) A long anterior elongation is present, composed of cells non-germinal in character and serving as a suspensory ligament. Homology with the "Endfaden" is uncertain.

(3) Ova are associated with nutritive cells that show distinct "yolk nuclei" at a certain stage.

(4) The germinal vesicle early becomes invisible and the nucleus does not again appear until after the polar bodies are given off.

(5) The egg is spherical, cleavage holoblastic at first and slightly unequal.

(6) There is a multipolar immigration suggesting gastrulation.

(7) Outer and middle germ layers are formed by migration, the entoderm remaining behind in the yolk with yolk cells.

(8) A precephalic organ homologous with the dorsal organ of some crustaceans, and the indusium of *Xiphidium* is developed in the early blastoderm stages.

(9) There are at least three cuticles formed during preblastodermic stages; two of the three are crenated.

<sup>1</sup> This figure is from an unpublished drawing of Dr. W. M. Wheeler, who kindly lent it for this purpose.

(10) The embryo appears encircling the egg as a narrow girdle, stopping each side of the precephalic organ.

(11) An extra pair of mouth-parts appears, forming in the adult two lateral folds inclosing the mouth-parts. This is homologous with the second pair of crustacean antennae.

(12) Yolk is included in the reproductive organs and lies free in the body cavity, but is not found in the mesenteron.

(13) *Anurida* shows characters allying it with crustaceans and myriapods rather than the rest of the Insecta.

In consideration of all these points it is clear that *Anurida* possesses certain characters allying it closely to the lower arthropod groups. The holoblastic cleavage and egg membranes ally it to both crustaceans and myriapods, while the structure of the ovary is most like the synthetic type *Scolopendrella*, but more like the chilognath myriapod than the chilopod. In spite of the possession of some generalized characters, it is evident that *Anurida* is a degenerate type that has been developed by a lengthening of embryonic life and a shortening of adult life. Paedogenesis, the sexual maturing of a larva, is illustrated by this process. The absorption of the embryonic yolk by the reproductive organs and the great maturity of the products even immediately after hatching both point to a tendency to shorten adult life and to omit larval development even to the extent of assuming the larval form for the adult. The decrease in the number of abdominal segments is only another step in the same direction. If the insect may be considered a larval chilognath sexually matured and bearing the three pairs of legs found in the chilognath larva, so can an *Anurida* be considered a very simple insect embryo matured sexually. Observations have been before advanced to establish the progressive shortening in some forms and gradual elimination of larval forms.

*Anurida* shows additional interesting points. By its curious habitat, chiefly under water, it has lost the need for tracheae, and, consequently, they are so far obliterated as to be absent even in the embryo; its respiration is purely cutaneous. It has been remarked that the amnion and serosa, the cellular envel-



opes of higher tracheates, are connected strictly with terrestrial forms and are one of the necessary adaptations to the exigencies of land life. Whether or not the ancestors of *Anurida* ever possessed such structures and have since lost them in consequence of acquired semi-aquatic life cannot be settled, but it is interesting to speculate on the possibilities of *Anurida* being a simple form and still retaining a semi-aquatic mode of life and showing a few transitional characters.

*Notes on Other Points of Interest.*

*Nervous System.* — No detailed observations were made on the development of the central nervous system, but a few points of correspondence with other forms were noted. The brain and ventral cord both arise in the same way as that described by Wheeler ('93) for *Xiphidium*, — by the proliferation from single ectoderm cells until rows of nerve cells arise. Proliferation is in the direction of the dorso-ventral axis of the embryo, and is restricted to certain places in the segments; subsequently, these primitive ganglia are united to form the ventral cord. Ultimately the six abdominal ganglia are fused to form a mass. The brain portion may be readily seen to have the three successive segments, protocerebrum, deutocerebrum, and tritocerebrum. The optic lobes form a large part of the young protocerebrum. Above the stomodeal invagination soon arose by proliferation from the hinder end a cord similar in structure to the ectoderm of the invagination that after reaching a considerable length remains unchanged, but a prominent character even in late embryonic life. It is entirely missing in the young animal and nothing remains to suggest its former presence. From the method of origin it is concluded that this is a trace of a sympathetic system, this being the history of this system in other insects; but since the adult seems to lack a sympathetic system, its degeneration is to be expected. *Pauropus*, that low, degenerate myriapod, also lacks a sympathetic system. These are mere notes on the general features of the nervous system, and a more complete study will be reserved for a future occasion.



*Respiratory System.*—Anurida, as has long been known, lacks entirely any tracheal system; respiration is carried on wholly by means of the skin. In the embryos no invaginations were seen to represent even the rudiments of such a system. There are, however, at the bases of the legs and at different parts of the abdomen large unicellular glands that may have some relations to tracheal openings or the different glands found in myriapods and other Tracheata. This point, too, remains for further investigation.

In conclusion I wish to acknowledge the helpful oversight given to me during this investigation by the Department of Zoölogy of the University of Chicago, where the greater part of the work was done. My thanks are especially due to Dr. W. M. Wheeler, under whose direct supervision the subject was undertaken. I am also indebted to him for many valuable suggestions in the treatment of the subject and in methods and much assistance in reaching literature, and I wish here to express my grateful appreciation of the aid so freely given.

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Since finishing this article, two contributions have been made to our knowledge of the development of the Apterygota, in both cases of the Thysanura. One article is by Dr. Heinrich Uzel, in the *Zoologischer Anzeiger* (Bd. XX, Nrs. 528, 529, and 535) for 1897, entitled "Beiträge zur Entwicklung der Thysanuren" (*Campodea staphylinus* Westw. and *Lepisma saccharina* L.). Another is by Dr. R. Heymons, published in the *Zeitschrift für wissenschaftliche Zoologie* for 1897, on the subject of "Entwicklungsgeschichtliche Untersuchungen an *Lepisma saccharina*, L." There are many points of interest between the observations made by these authors on the Thysanura and those given above for Anurida; one or two in particular will be briefly mentioned.

Both authors describe the cleavage as distinctly superficial in *Lepisma*, and Uzel observes the same to be true in the spherical egg of *Campodea*. This is an interesting point in

consideration of the sizes and shapes of the eggs in the three forms. In *Lepisma* the egg is a regular oval, and about 1 mm. in its longest diameter; in *Campodea* the egg is spherical, and has a diameter of about 0.4 mm., while the egg of *Anurida* has also a spherical form, but is only about 0.27 mm. in diameter. This increase in size is enough to explain the loss of holoblastic cleavage in the larger forms, considering its imperfect preservation in the small anuridan egg.

The process of germ band formation as described by Uzel for *Campodea* agrees very closely with the corresponding one in *Anurida*. A "dorsal organ" is described as having a position comparable to that held by the precephalic organ in *Anurida*. There are in *Campodea* no embryonic membranes corresponding to the amnion and serosa of the pterygote insect. In *Lepisma* these structures appear, but the amniotic sac remains open for a short distance. In many ways *Campodea* suggests to Uzel the myriapod type of development.

In the more complete consideration of *Lepisma* by Heymons there are several special points of interest. He finds rudimentary appendages upon the tritocerebral segment, which eventually disappear in early embryonic life. In discussing the origin of the mesenteron, several observations agree closely with those made on *Anurida*. The appearance of this part of the alimentary tract is very much delayed. Not until after hatching is it definitely formed. Unfortunately, certain critical stages were not found; but the author saw in late embryonic stages small groups of cells taking up a peripheral position on the yolk. These groups increase by rapid division originating from what the author considers yoke cells that have been functioning throughout embryonic life in the assimilation of yolk. Hence he says the mesenteron is truly entodermic in origin. There is only one principal point of difference between this view and the one given for *Anurida*. In the latter case, it is clear that the mesenteron arises from cells originating at the same time as the yolk cells, but remaining latent through the early embryonic stages; the yoke cells themselves degenerate at the close of embryonic life. Possibly there is some such latent source in *Lepisma* that may have escaped observation.

The origin of the germ cells is another question of extreme interest. It was only after prolonged study that the interpretations of the facts observed in *Anurida*, as given above, were formulated. One point observed in *Lepisma* corroborates in part the conditions described in *Anurida*. It is clear that, as in *Blatta*, the germ cells are subject to great changes of position. According to Heymons they have an ectodermal origin, and appear early in embryonic life. The formation of egg tubes and their connection with each other are all steps accomplished by the process of migration. There is clearly nothing metameric in their origin, and any such arrangement must be secondary. At present nothing further can be said on the diverse origins of the germ cells, — from the ectodermic in one case and mesoderm in the other.

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<i>a<sub>1</sub>-a<sub>6</sub></i> .	abdominal segments	<i>m.g.c.</i>	migrating germ cells.
<i>an.</i>	anus.	<i>mp.</i>	mouth-parts.
<i>a.o.</i>	abortive ovum.	<i>m.pr.</i>	male pronucleus.
<i>at.</i>	antenna.	<i>mx<sub>1</sub>.</i>	} maxillae.
<i>b.c.</i>	blood corpuscle.	<i>mx<sub>2</sub>.</i>	
<i>bl.</i>	blastomeres.	<i>n.</i>	nucleus.
<i>c<sub>1</sub>.</i>	} crenated membranes.	<i>n.c.</i>	nutritive cells.
<i>c<sub>2</sub>.</i>		<i>ncl.</i>	nucleolus.
<i>c.b.</i>	central blastomeres.	<i>nr.</i>	nervous system.
<i>c.el.</i>	cephalic elongation.	<i>o.</i>	ovum.
<i>c.g.</i>	cavity of mid-gut.	<i>o.d.</i>	oblique division.
<i>ch.</i>	chromatin.	<i>ov.</i>	ovary.
<i>cl.</i>	collophore.	<i>p.</i>	preparatory membrane.
<i>c.p.</i>	central mass of protoplasm.	<i>p.b<sub>1</sub>.</i>	} polar bodies.
<i>dc.</i>	deutocerebrum.	<i>p.b<sub>2</sub>.</i>	
<i>e.</i>	eye.	<i>p.c.</i>	protocerebrum.
<i>ec.</i>	ectoderm.	<i>p.c.o.</i>	precephalic organ.
<i>e.m.</i>	egg membrane.	<i>p.d.</i>	proctodaeum.
<i>en.</i>	entoderm.	<i>p.i.</i>	protoplasmic island.
<i>f.</i>	follicle.	<i>p.n.</i>	protoplasmic network.
<i>f.g.</i>	fat globules.	<i>r.</i>	radial protoplasm.
<i>f.pr.</i>	female pronucleus.	<i>r.o.</i>	opening for reproductive organ.
<i>g.c.</i>	germ cell.	<i>s.</i>	sperm cells.
<i>g.e.</i>	germinal epithelium.	<i>s.g.c.</i>	stationary germ cells.
<i>g.v.</i>	germinal vesicle.	<i>sp.me.</i>	splanchnic mesoderm.
<i>i.c.</i>	intercalary segment.	<i>st.</i>	stomodaeum.
<i>int.</i>	intestine.	<i>t<sub>1</sub>.</i>	} thoracic segments.
<i>k.</i>	knob.	<i>t<sub>2</sub>.</i>	
<i>l.m.</i>	longitudinal muscle.	<i>t<sub>3</sub>.</i>	
<i>lb.</i>	labrum.	<i>t.a.</i>	thoracic appendages.
<i>m.</i>	membrane.	<i>tc.</i>	tritocerebrum.
<i>mc.</i>	muscles.	<i>y.</i>	yolk.
<i>md.</i>	mandibles.	<i>y.c.</i>	yolk cells.
<i>me.</i>	mesoderm.	<i>y.m.</i>	yolk mass.
<i>me.s.</i>	mesoblastic somite.	<i>y.n.</i>	yolk nucleus.
<i>m.f.</i>	mouth fold.	<i>v.</i>	vitelline membrane.





## EXPLANATION OF PLATE XX.

NOTE. Unless otherwise stated drawings are made with Zeiss lenses and the ordinary tube length. Objective  $\frac{1}{2}$  is an oil immersion.

*Anurida maritima* Guen., Figs. 1-13; stained in lithium carmine and Lyons blue, Fig. 14. *Tomoceras* sp.?

FIG. 1. Longisection of adult ovary in early summer. *cel.*, cephalic elongation attached to the fat body; *o.*, young ova; *n.c.*, nutritive cells associated with ova; *g.e.*, germinal epithelium; *ov.*, ovarian wall. Obj. 4, oc. 4.

FIG. 2. Group of germ cells from near the germinal epithelium, showing the characteristic way of association. Letters as in Fig. 1. Obj.  $\frac{1}{2}$ , oc. 6.

FIG. 3. Ovum at later stage, when the cytoplasm is increased. Letters as above. Obj.  $\frac{1}{2}$ , oc. 6.

FIG. 4. Section of ovary in younger stage than represented in Fig. 1, yolk not yet appearing. *p.n.*, cytoplasm of ovum, highly vesicular; *y.n.*, yolk nucleus of nutritive cells; *f.*, beginning of follicle. Obj.  $\frac{1}{2}$ , oc. 4.

FIGS. 5 and 6. Transections through cephalic elongation, showing non-germinal character of cells. Obj.  $\frac{1}{2}$ , oc. 4.

FIG. 7. Longisection through ovary in region of beginning of cephalic elongation. Letters as above. Obj.  $\frac{1}{2}$ , oc. 2.

FIG. 8. Young ovum and nutritive cells. Letters as above. Obj.  $\frac{1}{2}$ , oc. 6.

FIG. 9. Enlarged view of one of the small masses shown in Fig. 1. Letters as in 8. Obj.  $\frac{1}{2}$ , oc. 6.

FIG. 10. Enlarged view of an egg mass from ovary shown in Fig. 1. *ch.*, chromatin; *ncl.*, nucleolus; *n.*, nucleus of nutritive cells and ovary; *a.o.*, abortive ovum; *y.*, yolk appearing in ovum. The nucleus is already invisible. Obj.  $\frac{1}{2}$ , oc. 4.

FIG. 11. Late stage of ovarian development, showing parts of two eggs. Letters as above. Obj.  $\frac{1}{2}$ , oc. 2.

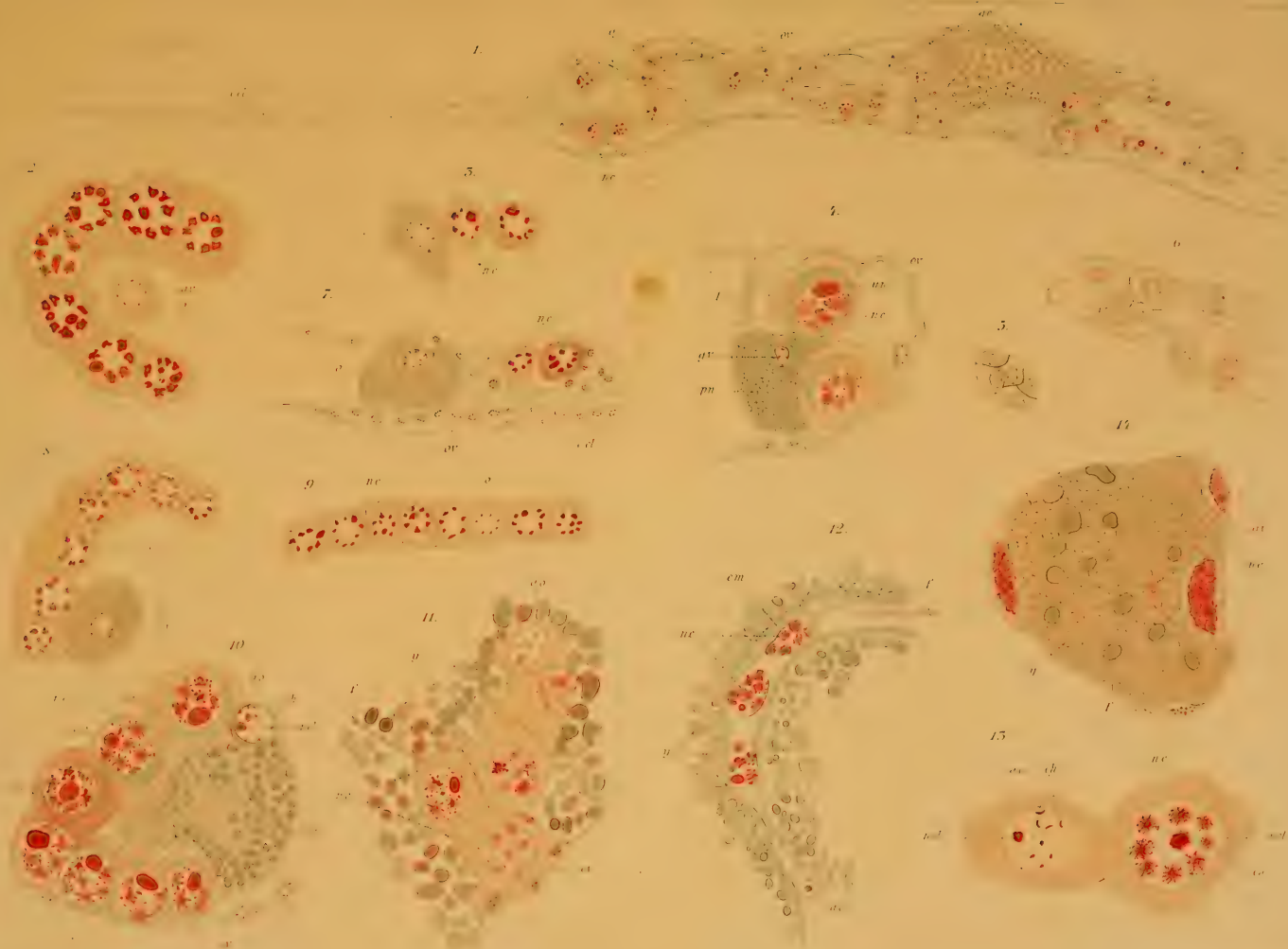
FIG. 12. Final stage in ovarian development. *em.*, egg membrane. Obj.  $\frac{1}{2}$ , oc. 2.

FIG. 13. Enlarged view of abortive ovum and nutritive cell. *ncl.*, nucleolus; rest of letters as in Fig. 10. Obj.  $\frac{1}{2}$ , oc. 4.

FIG. 14. Ovum from ovary of Thysanuran (*Tomoceras*), showing the beginning of yolk formation and the preservation of the group of chromosomes. Letters as before. Obj.  $\frac{1}{2}$ , oc. 3.













## EXPLANATION OF PLATE XXI.

*Cleavage.*

FIG. 15. External view of unsegmented eggs, showing grouping. Obj. 4, oc. 2.

FIGS. 16-24 are surface views of cleavage stages, showing the 2, 4, 8, 16, 32, and coarse morula stages until total cleavage ceases. Drawn from unstained eggs. Obj. 16, oc. 6.

FIG. 25. Protoplasmic mass at the surface of egg. *f.pr.*, female pronucleus returning to centre; *p.b.*, polar bodies. Borax carmine. Obj.  $\frac{1}{12}$ , oc. 6.

FIG. 26. Showing division of first polar body. Letters as in Fig. 25. Borax carmine. Obj.  $\frac{1}{12}$ , oc. 6.

FIG. 27. Part of protoplasm from the centre of the unsegmented egg. *f.pr.*, female pronucleus; *m.pr.*, male pronucleus. Borax carmine. Obj.  $\frac{1}{12}$ , oc. 6.

FIG. 28. First cleavage spindle. Iron haematoxylin and Orange G. Obj.  $\frac{1}{12}$ , oc. 2.

FIG. 29. Reconstruction of nucleus after division into the 2-cell stage. Letters as before. Iron haematoxylin and Orange G. Obj. 4, oc. 4.













## EXPLANATION OF PLATE XXII.

*Blastoderm Formation.*

FIG. 30. Section through unsegmented egg. *p.i.*, protoplasmic island in which female pronucleus is present; *c.p.*, central mass of protoplasm; *r.*, radial protoplasmic strands; other letters as before. Borax carmine. Obj. 4, oc. 6.

FIG. 31. Section through line Z-Z in Fig. 21. *bl.*, blastomere; *e.m.*, egg membrane; *v.*, vitelline membrane. Iron haematoxylin and Orange G. Obj. 8, oc. 6, tube length 15 $\frac{1}{2}$ .

FIG. 32. 32-cell stage. *c.b.*, central blastomeres. Erlich's haematoxylin. Obj. 4, oc. 6.

FIG. 33. Part of egg after holoblastic cleavage has ceased. *bl.*, blastomeres from which nuclei surrounded by protoplasm are migrating. Borax carmine. Obj. 4, oc. 6.

FIG. 34. Early blastoderm. *ec.*, ectoderm; *me.*, mesoderm; *y.m.*, yolk masses showing earlier position of blastomeres; *o.d.*, oblique division of ectoderm cells. Erlich's haematoxylin. Obj. 6, oc. 6.

FIG. 35. Blastoderm formation completed. *pc.o.*, beginning of the precephalic organ; *en.*, entoderm cells; *y.c.*, yolk cells; the rest as in Fig. 34. Borax carmine. Obj. 8, oc. 6.

FIG. 36. Later stage of blastoderm. Letters as in Fig. 35. Borax carmine. Obj. 8, oc. 6.

FIG. 37. Still later stage. Letters as in Fig. 35. Borax carmine. Obj. 4, oc. 1.

FIG. 38. Precephalic organ at its period of greatest development, blastoderm crenated. *c<sub>1</sub>.*, first crenated membrane; remaining letters as before. Borax carmine. Obj. 4, oc. 1.

FIG. 39. Precephalic organ elongated. *c<sub>2</sub>.*, second crenated membrane; *k.*, knob where elongation is attached to crenated membrane number 2; remaining letters as above. Borax carmine. Obj. 4, oc. 1.

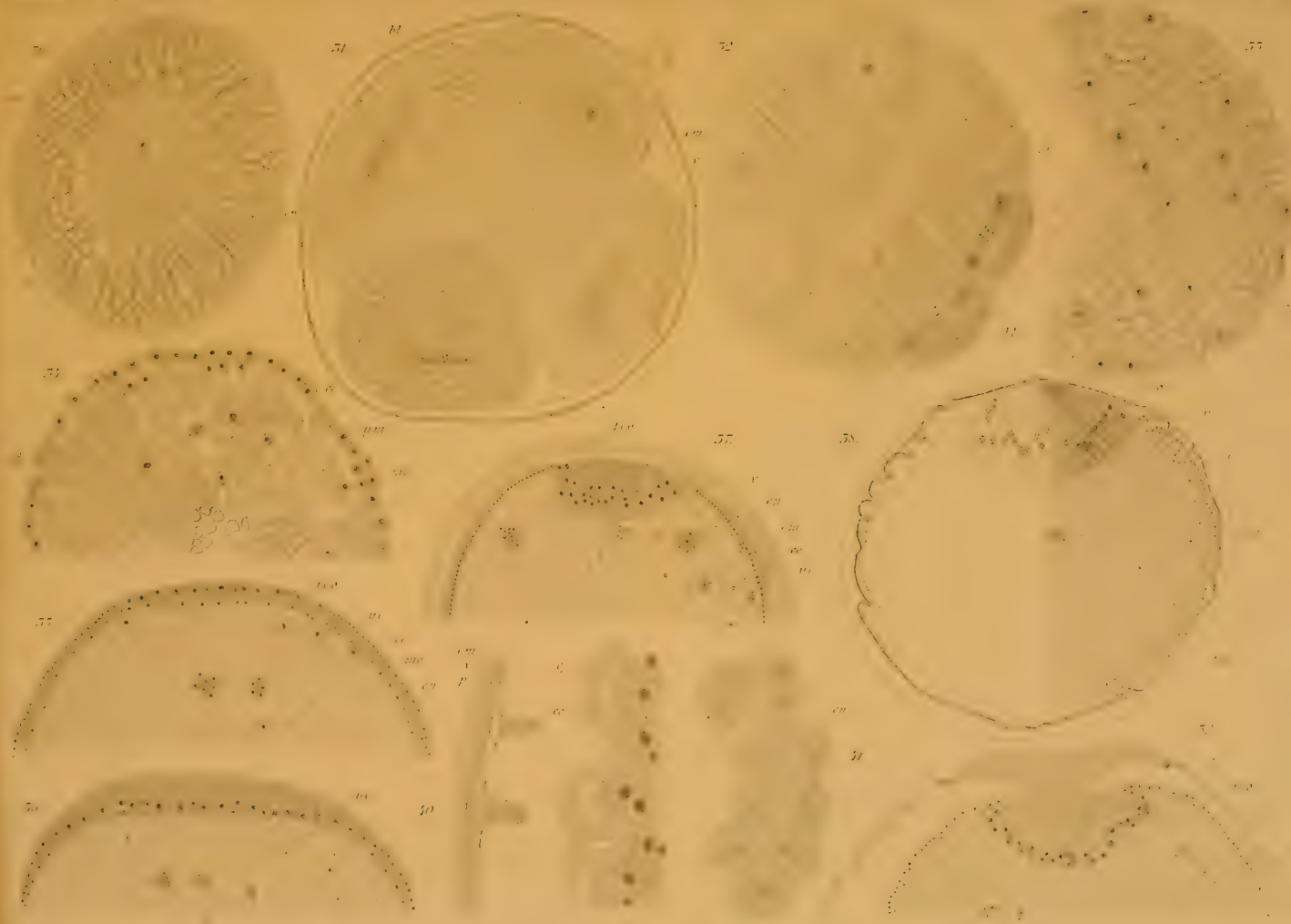
FIG. 40. Enlarged view of section of membranes and ectoderm of ovum. *p.*, preparatory membrane; other letters as before. Borax carmine. Obj. 1 $\frac{1}{2}$ , oc. 6.

FIG. 41. Entoderm cells showing vesicular protoplasm. Borax carmine. Obj. 1 $\frac{1}{2}$ , oc. 4.













## EXPLANATION OF PLATE XXIII.

*Surface Views of Embryos.* Erlich's Haematoxylin.

FIG. 40. Ventral view of early embryo. The embryo is rolled over and represented as laid out flat. *at.*, antenna, *i.c.*, intercalary appendage (2d antenna); *md.*, mandibles; *mx<sub>1</sub>*, *mx<sub>2</sub>*, maxillae; *t<sub>1</sub>*, 1st thoracic legs; *pc.o.*, precephalic organ. Obj. 8, oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 41. *t<sub>2</sub>*, *t<sub>3</sub>*, 2d and 3d thoracic legs; *a<sub>1</sub>*, 1st abdominal appendage; other letters as above. Obj. 8, oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 42. *lb.*, labrum; *a<sub>1</sub>-a<sub>5</sub>*, abdominal segments and beginning of appendages; *pd.*, proctodaeum; remaining letters as in Fig. 41. Obj. 8, oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 43. Face view of later embryo, shown in side view in 43*a*. Flexure just beginning. *mf.*, mouth fold; *m.p.*, mouth-parts; *ta.*, thoracic appendages; *cl.*, colophore; *a<sub>2</sub>-a<sub>4</sub>*, appendages on abdomen. Obj. 4, oc. 6, tube length  $15\frac{1}{2}$ .

FIG. 43*a*. Outline side view of embryo represented in Fig. 43, showing beginning of flexure. Obj. 16, oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 44. *pc.o.*, precephalic organ elongating; *m.*, last membrane shed; other letters as above. Obj. 8, oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 45. Flexure almost complete. *e.*, eyes; remaining letters as before. *a<sub>4</sub>* is larger than any of other abdominal appendages excepting the colophore. Obj. 8, oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 46. Head of embryo showing the beginning of the mouth fold, *mf.* Obj. 8, oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 47. Enlarged head of embryo of same stage as Fig. 42. *pc.*, procerebrum; *dc.*, deutocerebrum; *tc.*, tritocerebrum bearing *i.c.*, the intercalary appendage. Obj. 8, oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 48. Newly hatched young *Anurida maritima*. Letters as before. Reichert, obj. 3, oc. 1.













## EXPLANATION OF PLATE XXIV.

*Development of the Reproductive Cells.*

FIG. 49. Transection showing early mesoderm formation. Letters as before. Borax carmine. Obj.  $\frac{1}{12}$ , oc. 2, tube length  $15\frac{1}{2}$ .

FIG. 50. Transection through somite on one side of body in embryo of age shown in Fig. 44, 2d abd. segment. *g.c.*, germ cells. Delafield's haematoxylin. Obj.  $\frac{1}{12}$ , oc. 6, tube length  $15\frac{1}{2}$ .

FIG. 51. A similar section of same age as in Fig. 50. *me.s.*, mesoblastic somite, cavity distinct. Borax carmine. Obj.  $\frac{1}{12}$ , oc. 6, tube length  $15\frac{1}{2}$ .

FIG. 52. Longitudinal section through under part of abdomen of later embryo. *mc.*, beginning of muscles; other letters as before. Delafield's haematoxylin. Obj.  $\frac{1}{12}$ , oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 53. Longisection through abdomen of stage shown in Fig. 45. *sp.me.*, splanchic layer of mesoderm; *bl.c.*, blood corpuscles; other letters as above. Delafield's hematoxylin. Obj.  $\frac{1}{12}$ , oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 54. Longisection through hinder part of abdomen of embryo in corresponding stage (slightly oblique). *an.*, anus; rest of letters as before. Germ cells are migrating into the yolk. Delafield's haematoxylin. Obj.  $\frac{1}{12}$ , oc. 4, tube length  $15\frac{1}{2}$ .

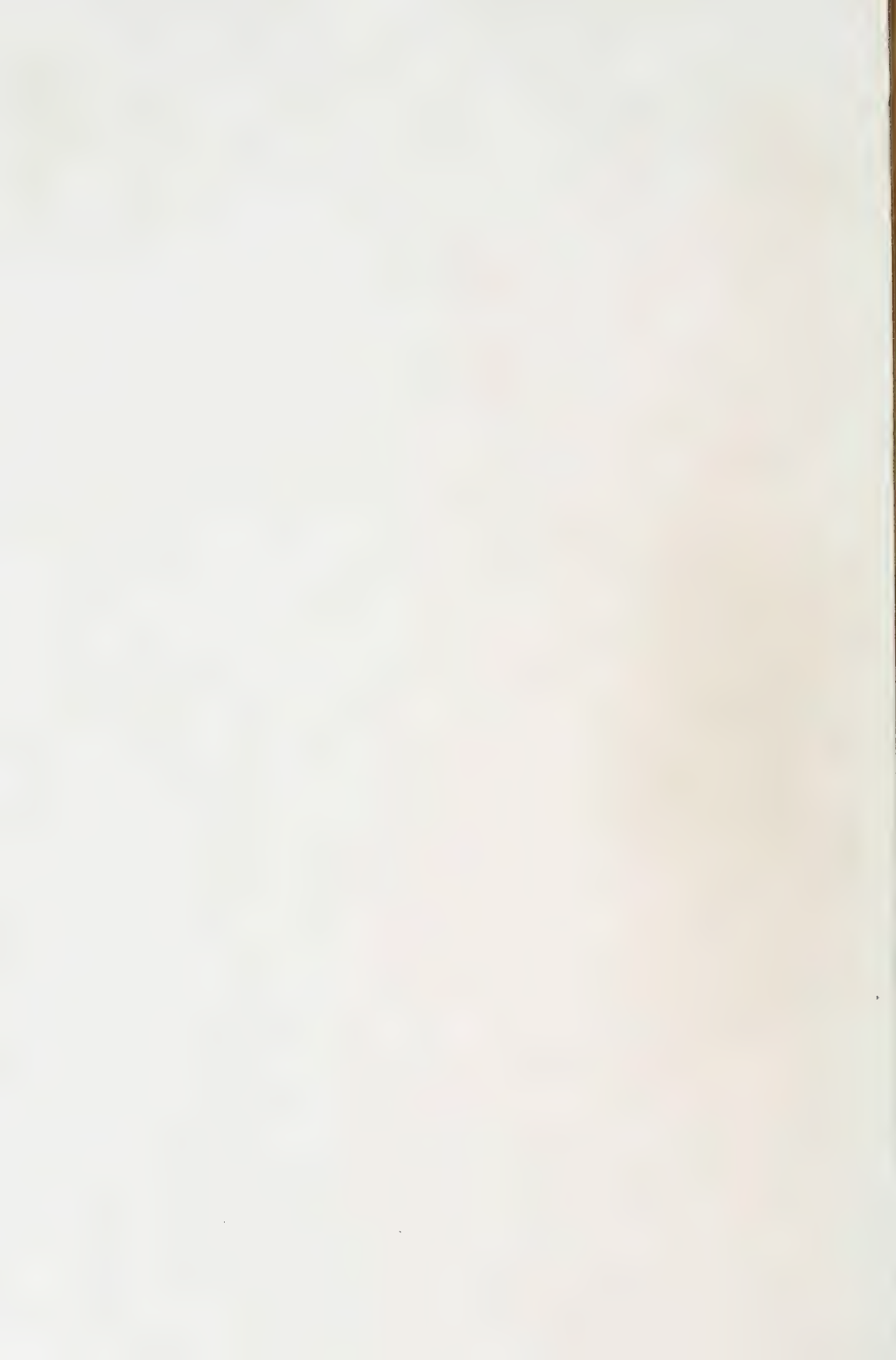
FIG. 55. Longisection through embryo. *me.*, two layers of mesoderm, splanchic and somatic. Delafield's haematoxylin. Obj.  $\frac{1}{12}$ , oc. 6, tube length  $15\frac{1}{2}$ .

FIG. 56. Similar section to Fig. 55. *s.g.c.*, stationary germ cells; *m.g.c.*, migrating germ cells. Delafield's haematoxylin. Obj.  $\frac{1}{12}$ , oc. 6, tube length  $15\frac{1}{2}$ .

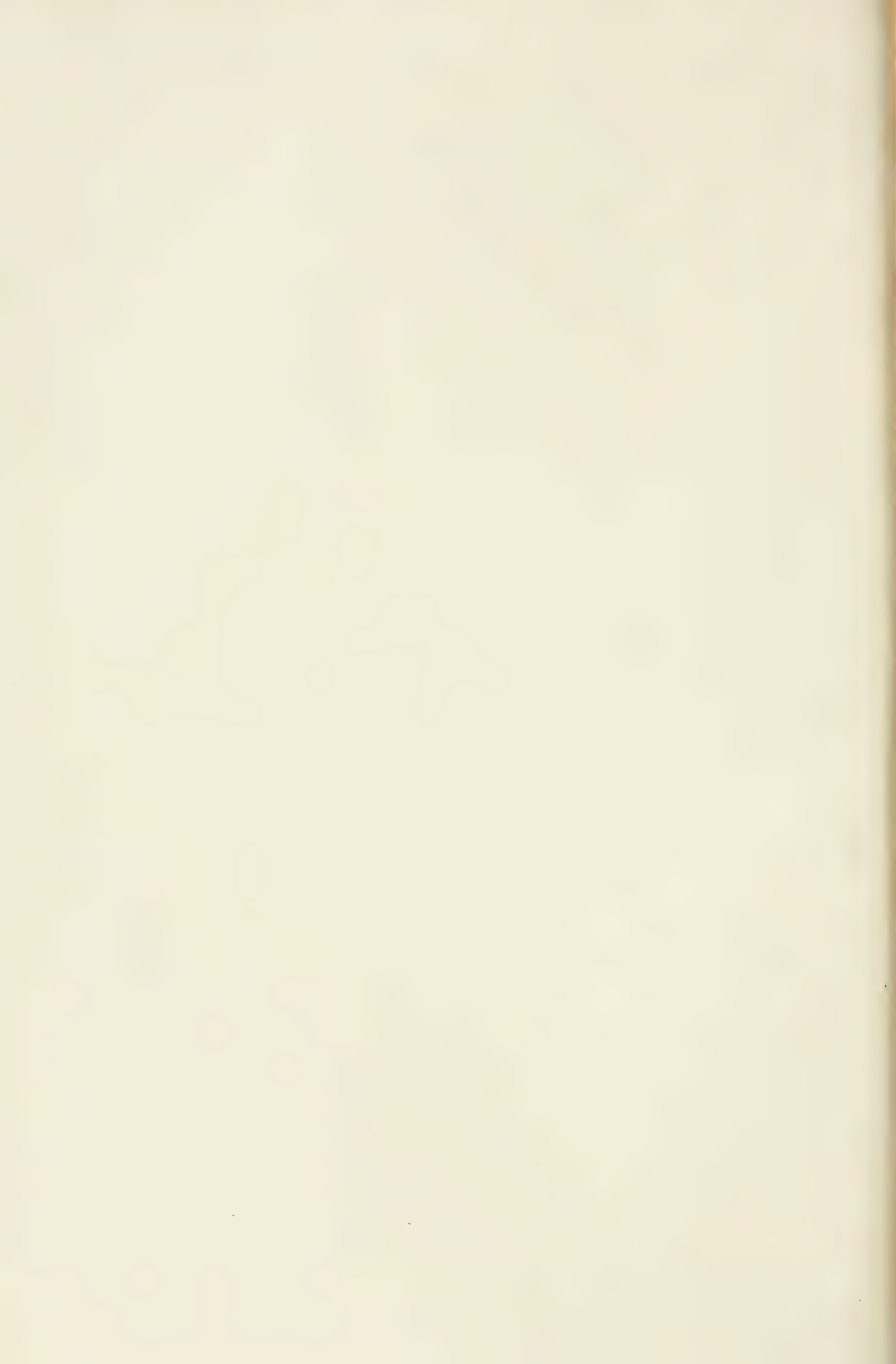
FIG. 57. Section showing migrated germ cells and scattered degenerating yolk nuclei, *y.c.* Obj.  $\frac{1}{12}$ , oc. 4, tube length  $15\frac{1}{2}$ .















## EXPLANATION OF PLATE XXV.

FIG. 58. Longisection through abdomen of last stage of embryo. *en.*, entoderm. Other letters as before. Borax carmine and Orange G. Obj.  $\frac{1}{2}$ , oc. 4, tube length  $15\frac{1}{2}$ .

FIGS. 59, 60, 62. Cross-sections of ovaries of just-hatched Anurida. *y.*, embryonic yolk; rest of letters as before. Borax carmine and Orange G. Obj.  $\frac{1}{2}$ , oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 61. Cross-section of small animal taken early in the summer. Erlich's haematoxylin. Obj.  $\frac{1}{2}$ , oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 63. Blood corpuscles from just-hatched animal containing yolk. Borax carmine. Obj.  $\frac{1}{2}$ , oc. 6, tube length  $15\frac{1}{2}$ .

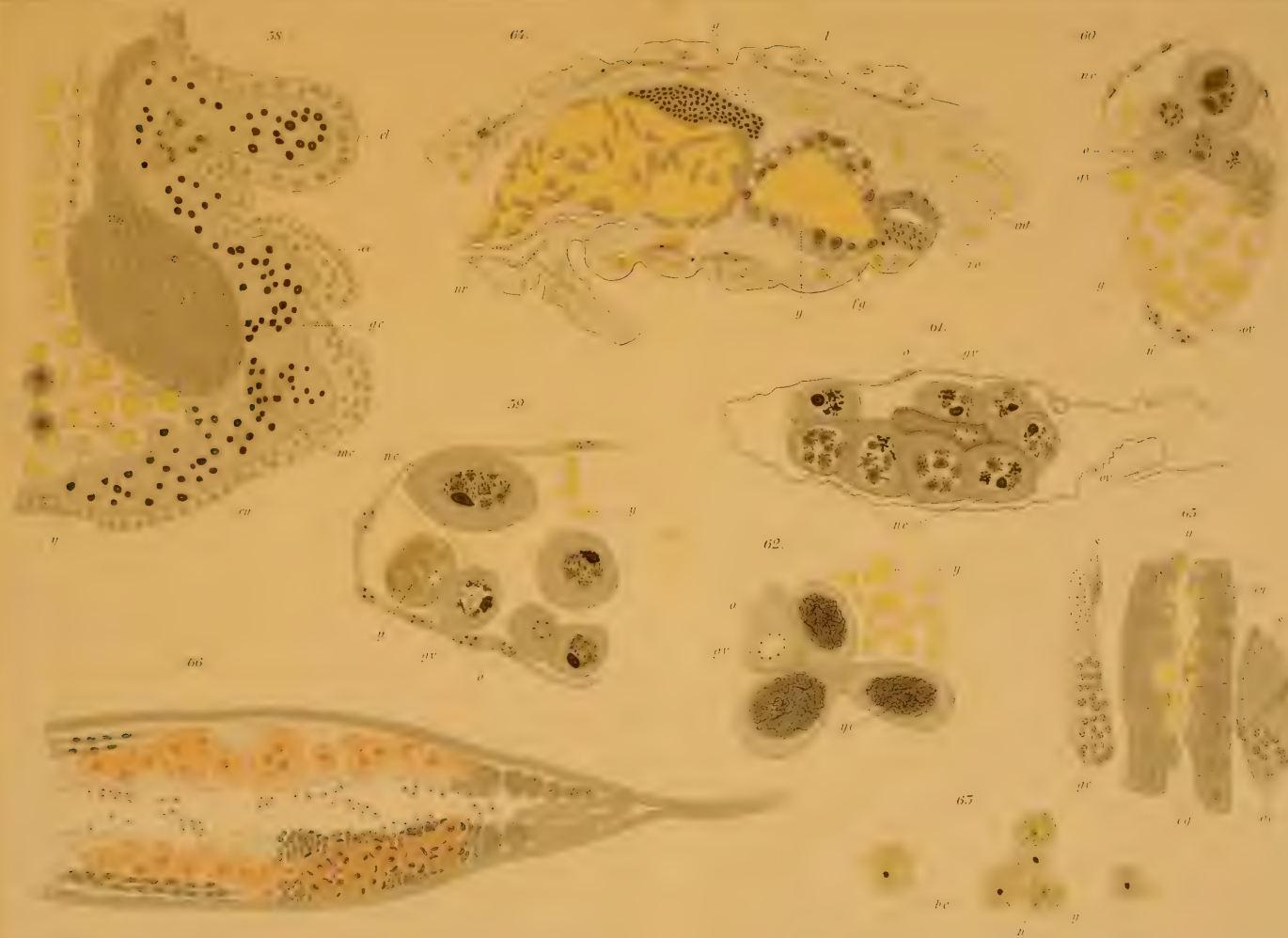
FIG. 64. Slightly oblique longisection of just-hatched male. *L.*, longitudinal muscles; *fg.*, fat globules; *int.*, intestine; *r.o.*, opening of reproductive organs; *n.c.*, nerve cord; other letters as before. Erlich's haematoxylin. Obj. 8, oc. 4, tube length  $15\frac{1}{2}$ .

FIG. 65. Longisection through mid-gut of just-hatched male. *en.*, entoderm; *y.*, yolk; *c.g.*, cavity of gut; *s.*, sperm cells; *g.e.*, germinal epithelium. Borax carmine and Orange G. Reichert, obj.  $\frac{1}{2}$ , oc. 1.

FIG. 66. Frontal view of Petromyzon from a drawing by Dr. W. M. Wheeler. This shows the association of yolk with germinal cells.











FORMATION OF THE GERM LAYERS IN THE  
AMPHIPOD MICRODEUTOPUS GRYLLO-  
TALPA COSTA.

CLARA LANGENBECK.

THE interesting results which Dr. McMurrich obtained from a study of the cytogenesis of the isopods led him to suggest to me that I should undertake the study of amphipod development from the same standpoint.

This investigation was begun under Dr. McMurrich's direction in the summer of 1893, at the Marine Biological Laboratory, Woods Holl, Mass., was continued there during the two following summers, and was completed during my term of the Biological Fellowship at Bryn Mawr College, in the winter of 1895-96. I wish here to express my thanks to Dr. McMurrich, as well as to Dr. Whitman, Director of the Marine Biological Laboratory, and to Dr. Morgan, Professor of Zoölogy, Bryn Mawr College, for their kind interest and assistance.

Up to the present time observations which have been recorded upon the segmentation stages of the amphipod ovum have been made only upon the living egg. For a historical sketch of the literature I refer the reader to the *Monograph upon the Amphipods*, by Della Valle ('93). The only paper upon the subject which has appeared since then was published in December of the same year by Bergh ('93), who calls attention to the interesting rotation of the embryo upon the egg, and points out the necessity of studying the whole cleared egg before sectioning it. The paper does not go into detail, being intended rather as a suggestion for future work than as an exposition of amphipod development.

My observations were made upon the egg of a small marine amphipod, *Microdeutopus gryllotalpa* Costa, which lives in shallow water among decaying seaweed. This species is widely distributed, being found on the coast of New England as well

as on the European coast from Norway to the Mediterranean.<sup>1</sup> It is most plentiful and accessible, and, as I was able to collect a complete series of embryos, it proved a very favorable species upon which to work. For a description of the animal I refer the reader to Della Valle ('93), who places it in family IV, Corophiidae. To this same family belong also *Amphitoë* and *Sunamphitoë*, whose development has been described by Mlle. Rossiiskaya ('91). Judging from the resemblance of her figures to those I have of *Microdeutopus*, the modes of development of these species must be almost identical; our interpretations, however, differ widely.

The Corophiidae are placed in the sub-order Crevettina together with the Gammaridae and the Orchestiidae (Leunis), families whose development has received the most attention. *Microdeutopus* itself was studied by Della Valle. In his introduction to the embryology he says he studied not only the eggs of *Orchestia* and *Gammarus*, which he figures and describes, but also amphipods of other families, especially *Microdeutopus gryllotalpa*, as control observations. Della Valle is convinced from his studies that, on the whole, there is no essential difference in the development of these groups. There are many points in his description which I am not able to bring into harmony with what I found for *Microdeutopus*, but I shall defer the discussion of these points until I have given my own results.

#### *Methods.*

*Microdeutopus* lives in shallow water among decaying seaweed. By taking small portions of the seaweed at a time and squeezing them the animals came out of hiding and could be easily caught. They were then placed in glass dishes with fresh salt water, and kept in captivity for several days. It is very difficult to catch the animals with eggs in the early stages, though why this should be so I cannot tell. Quantities of females with the eggs twenty-four hours old could be found

<sup>1</sup> Prof. Sidney I. Smith, of Yale University, who very kindly identified the amphipod, made the statement about its distribution in a letter to Dr. McMurich, to whom I am indebted for the information.

each day; but, although the seaweed was carefully searched, the number of eggs in the segmentation stages found in the material brought in on one day was out of all proportion to the number of eggs twenty-four hours old found in the material brought in the next day from the same place. The animals do not hide in the mud during the early period of development of their eggs, because the bottom of the pool in which they were collected was composed of a black refuse, which gave off so much marsh gas that the animals could not live in it. By carefully watching those kept in captivity, I observed that when there was a moulted amphipod shell floating at the surface of the water, an animal which had just deposited its eggs was almost always to be found in the dish, and in this way the early stages were obtained.

The females were caught and firmly held with a forceps while the eggs were removed from the brood pouch with a dissecting needle. The eggs were then killed in a modification of Kleinenberg's picro-sulphuric solution, in which sea water was substituted for the ordinary distilled water. This solution gave better results than the ordinary Kleinenberg killing fluid, which distends the egg. Corrosive sublimate, as suggested by Della Valle, also distends the egg and injures the protoplasmic structure. The living egg contains a fluid substance which exudes into the space between the surface of the egg and the chorion as soon as the egg is killed. This fluid substance coagulates and is stained by haematoxylin, the stain, however, being extracted by the acid alcohol before the protoplasm is decolorized. I could find no killing fluid which would prevent this exudation. Hot corrosive sublimate, Perenyi's, Flemming's, and Kleinenberg's fluids, alcoholic picro-sulphuric acid, and hot water all affected the egg, in this respect, in the same way. With the modified Kleinenberg solution the eggs shrink considerably, but the parts are not distorted, and good, clear nuclear figures were always obtained. The protoplasm showed no abnormal vacuolization, such as occurred when corrosive sublimate was used.

The chorion closely invests the fresh egg, but in the killed specimen there is a large space between it and the surface of

the egg. It was, therefore, found advisable to dissect off the chorion, since it collapses when the egg is placed in oil of cloves, and the resulting folds in it are easily mistaken for cleavage furrows.

The eggs were overstained in Kleinenberg's or Delafield's haematoxylin, washed out with acid alcohol, dehydrated, cleared in oil of cloves, and mounted under a cover slip supported by wax feet. By pushing the cover slip from side to side the eggs could be rolled into any desired position while they were being studied. Mlle. Rossiiskaya attempted to work upon the whole egg viewed as a transparent object, but says that she met with no success. I found that, unless a strong condensing lens is used, it is, as Mlle. Rossiiskaya ('88) says, almost impossible to distinguish the cellular structure, but with the condensing lens the cells can be distinctly and clearly seen. These same eggs which had been studied *in toto* were then imbedded in paraffin and sectioned. It was not possible to cut the segmentation and early blastoderm stages thinner than 10  $\mu$ , because the protoplasm at this time is so distributed that it does not seem to offer sufficient support to the yolk. Sections of the later stages were cut 5  $\mu$  thick, and preferably stained upon the slide. Eggs of the third day and after, when the yolk was partly digested, were stained with a  $\frac{1}{2}\%$  aqueous solution of haematoxylin and washed out in iron alum, according to the usual iron-alum method. This stain could not be used for the earlier stages, because the undigested yolk becomes very black and totally obscures the structure of the egg.

I tried to orient the eggs for sectioning according to Patten's method. They were so small, however, that the least amount of celloidin which would hold them to the paper formed a coat over the eggs so that the paraffin did not penetrate. Knowing the relative position of the dorsal organ with respect to the rest of the embryo, from a study of the cleared egg, it was not difficult to orient sections.



*Segmentation.*

A complete account of the cell genesis of the amphipod egg has never been published. The segmentation has been followed only on the living egg. The most complete account was published by Van Beneden and Bessels in 1869, who carry their observations up to the time when the blastoderm begins to appear upon the surface of the egg. Other authors merely state that the segmentation is total; that the third cleavage plane divides the egg unequally; that after the 32-cell stage the segmentation becomes irregular; that just before the blastoderm appears on the surface of the egg the difference in size between the micromeres and macromeres is lost; and that the protoplasm rises to the surface and the cells migrate toward the micromere pole to form the blastoderm. In the present work the segmentation was followed on the living egg as far as the 80-cell stage, and eggs in all stages of development were also studied as transparent objects. The drawings were all made from stained and cleared eggs, which were afterwards imbedded in paraffin, cut as described above, and studied in section.

I have never seen the process of fertilization in *Microdeutopus*, but I have caught many pairs of a closely allied amphipod in the act of copulation. In these forms the male rests upon the dorsum of the female, clasping her with the large chelae. He probably assists the female to slough. Sometimes the sloughing takes place soon after the animals have come together, and sometimes I have seen them united for days before the female sloughed. Shortly before she sheds her shell the male leaves her, and as soon as it is shed he returns, and the animals unite in the same way that they did before the sloughing took place. They remain together a short time and then separate, and shortly after the male has left for the second time the eggs are extruded. I do not know how nearly the process of fertilization in *Microdeutopus* agrees with what I have observed for the other amphipod, but a female of *Microdeutopus* which had not sloughed, but whose eggs were just in a condition to be extruded, was isolated. After a time she sloughed, and the eggs were extruded in the



normal way; but the eggs, although apparently quite normal, evidently were not fertilized, because they did not segment. I concluded, therefore, that in *Microdeutopus*, as in the other amphipod, fertilization takes place between the time of sloughing and the time of extrusion of the eggs. It was also observed that when a moulted amphipod shell was found floating at the surface of the water a female which had lately extruded her eggs was almost always in the dish.

When the eggs are first extruded into the brood pouch they are of a bright opaque green. The chorion closely invests the egg, but no other membrane could be seen either in the fresh specimen or in the sections. The eggs seem to be covered with some sticky substance, which causes those coming from a single ovary to cling to one another; but the groups of eggs from the two ovaries are separate. This substance is subsequently either absorbed or loses its sticky properties, because after the first cleavage the eggs separate readily as soon as they are removed from the brood pouch. The protoplasm is found at the center of the egg. It is irregular in outline, sending out long pseudopodia-like prolongations, which ramify throughout the egg, very much as Dr. McMurrich ('93) has shown to be the case in the egg of *Jaera*. No protoplasmic layer could be seen around the periphery of the egg, however; if it is there, it must be very thin. Fig. 19, which represents an egg passing from the 2-cell into the 4-cell stage, shows the manner in which the protoplasm ramifies throughout the yolk mass. The nucleus is found in the center of the protoplasmic area.

The segmentation in the early stages is total, but not equal; later it is superficial. The protoplasm loses its control over the inner ends of the blastomeres as it moves nearer the surface in the succeeding divisions, and the inner ends of the blastomeres fuse, so that the blastocoel, which is at first present, is obliterated.

*2-cell stage.* — About three hours after the eggs have been extruded into the brood pouch, the protoplasm in the center of the egg divides, the nuclear spindle lying in the long axis. After the two halves of the central protoplasm have separated a furrow appears at the surface of the egg and gradually deepens,

dividing the egg into two equal parts. The two blastomeres then flatten against each other, and the living egg presents the same form as it did before cleavage. I have never seen the blastomeres unequal in size at this stage, as Van Beneden and Bessels and Della Valle describe for the gammarids.

*4-cell stage.* — One hour elapses before the completion of the second division. Fig. 19 represents an optical section of an egg which was killed half an hour after the first division. The protoplasm has almost divided and the second cleavage furrow has begun to appear. This cleavage plane makes an acute angle with the first plane, giving rise to two small and two large blastomeres, the smaller blastomeres being even less than one-half the size of the larger ones, as shown in Fig. 1. When the egg comes to rest the two large cells flatten against each other, pushing the smaller ones apart in such a way that one lies above and the other below the plane of the equator, this plane being supposed to pass through the long axis of the egg and at right angles to the first and second cleavage plane; *i.e.*, in Fig. 1 it lies in the plane of the paper. No rotation of the blastomeres, as described by Wagner for *Melita* ('91), has ever been observed; the blastomeres always flatten against one another without changing their position. I always find, at this stage, two cells smaller than the other two, and not three of the same size and one somewhat smaller, as in the gammarids described by Van Beneden and Bessels ('69) and Della Valle ('93). Sometimes one of the two smaller blastomeres is larger than the other small one, but never as large as the large ones.

For convenience in describing the later stages, I shall name the larger cells *AB* and *CD*, the small cell above the equator *EF*, and the remaining one *GH*.

*8-cell stage.* — At the end of the fifth hour an equatorial furrow divides the egg into four micromeres and four macromeres (Fig. 2). The four micromeres bear to each other the same relation in size as the four macromeres, and the larger micromeres are smaller than the smaller macromeres. When the egg comes to rest the two large macromeres flatten against each other, and likewise the two large micromeres, while the two small macromeres and the two small micromeres are forced

apart. The larger micromeres stand just above the larger macromeres, and the same holds true for the smaller micromeres and macromeres. In the figures the macromeres and their descendants will be designated by the large letters, and the micromeres and their descendants by the small letters. The macromeres *AB*, *CD*, and *EF* and their descendants are drawn in red ink, while *GH* and the micromeres are in black.

From this time the *macromeres always divide before the micromeres*, and the larger macromeres before the smaller ones. The subsequent cleavage planes which divide the two larger macromeres are alternately meridional and equatorial, while the planes dividing *EF* and the micromeres are always meridional. This may be due to mechanical causes; since these blastomeres, lying upon the larger ones, are somewhat flattened, as shown in Fig. 25, the spindle would find more room in the horizontal than in the vertical plane.

*16-cell stage.*—After the sixth hour two vertical cleavages at right angles to each other give rise to *A, B, C, D, E, F, G, H, a, b, c, d, e, f, g, h* (Figs. 3, 4). When the egg comes to rest *A* and *C* flatten against each other, but *B* and *D* are forced apart by *G* and *H* (Fig. 3). It is to be noticed that the zone of small cells lies obliquely over the oval egg. Mlle. Wagner ('91) finds the same oblique zone of small cells in *Melita*, but the obliquity was, in that form, brought about by a rotation of the cells in the 4-cell stage. Her account agrees in part with the results of Van Beneden and Bessels ('69), whose Figs. 10, 11, and 12 show exactly the same oblique arrangement. In the text Van Beneden and Bessels make no mention of this oblique zone of cells, but in their Fig. 9, which represents the 8-cell stage, the cells are arranged symmetrically with reference to the long axis of the egg, while in their Fig. 10, where the macromeres are just beginning to pass into the 16-cell stage, the oblique position of the micromeres is manifest; therefore, it would seem that a rotation occurred just at this time. In *Microdeutopus* the obliquity is occasioned by the angle which the second cleavage plane makes with the first (Fig. 19).

*22-24-cell stage.* — Figs. 5-8 show four views of an egg of the 22-cell stage, Fig. 5 representing the macromere pole of the egg. In this stage *A*, *B*, *C*, *D*, *G*, and *H* have given rise to  $A^a$ ,  $A^b$ ,  $B^a$ ,  $B^b$ , etc., by an equatorial cleavage (Fig. 8), while *E* and *F* are still in the act of dividing and the micromeres show no trace of division.

*30-cell stage.* — Figs. 22-24 show three views of an egg of thirty cells passing into the 42-cell stage. The cells *E* and *F* have given rise to  $E^a$ ,  $E^b$ ,  $F^a$ , and  $F^b$  (Fig. 22). The cells *a* and *c*, *b* and *d* have all divided, forming  $a^b$ - $a^a$ ,  $c^a$ - $c^b$ , and  $b^b$ - $b^a$ ,  $d^a$ - $d^b$ , while  $g^a$  and  $h^a$ ,  $x$  and  $y$  are the descendants of *g* and *h*. Instead of the division being by an equatorial plane in these cells, as it is in the macromeres, and as Van Beneden and Bessels ('69), Rossiiskaya ('90), Della Valle ('93), and Ulianin ('81) found it for the micromeres of other amphipods, it is vertical and at right angles to the last plane of division of these cells, which also was vertical (Figs. 3, 4). That the division of the micromeres at this time is vertical and not equatorial in *Microdeutopus* is shown by the spindles in *g* and *h* (Fig. 11). I have also seen spindles in *b* and *d* lying in the equatorial plane and at right angles to those shown in *g* and *h*; *i.e.*, in the direction of the arrows in the cells *b* and *d* (Fig. 11). Figs. 11, 12 show the protoplasm in  $A^a$ ,  $A^b$ ,  $B^a$ ,  $B^b$ ,  $C^a$ ,  $C^b$ ,  $D^a$ ,  $D^b$  divided and the yolk deeply constricted; the nuclei of  $G^a$ ,  $H^a$  in the aster, and of *g*, *h* in the diaster stage. In Figs. 24, 25  $G^b$ ,  $H^b$  are beginning to divide; in  $G^a$ ,  $H^a$  the process is further advanced, and *g* and *h* have divided completely. The daughter cells of *g* and *h* I shall name  $g^a$ ,  $h^a$ ,  $x$ , and  $y$ , the  $x$ ,  $y$  cells being those lying next to  $b^b$ - $b^a$ ,  $d^a$ - $d^b$ . These are always present before  $G^a$ ,  $H^a$  have divided, although *e* and *f*, the cells corresponding to *g* and *h*, upon the other side, show no trace of division until a much later period (about the 72-cell stage). It is interesting to note that the cells of the *EF* and *ef* groups divide later than those of the *GH* and *gh* groups. It may be that the *EF* cell corresponds to the smaller cell which Della Valle ('93) found to result from the second division in *Orchestia*. He describes this cell as lagging behind the others in development.



At this stage  $x$  and  $y$  appear smaller and at a lower level in surface view, as though they were being pushed inward (Fig. 13). Sections just after this time (Figs. 9, 10) show forty-three cells arranged around a blastocoel, within which, at one end, lie two cells  $x^s, y^s$  (Fig. 9). The nucleus in one of these cells may still be seen (Fig. 9); in the other it is no longer present. I have also sections of one egg (Fig. 20) showing forty-one cells arranged around a blastocoel, and two of the cells have spindles whose equatorial planes are parallel to the surface of the egg. (Only one of these is shown in the figure.) The question now arises, Do the *two cells which are, after this stage, ALWAYS found in the center of the egg*, go in by a division parallel to the surface, or are they  $x$  and  $y$  which have been pushed in during the later division of the other cells? Unfortunately, I was not able to tell whether the two cells dividing inwards (Fig. 20) were the cells  $g, h$  or not. Although I have cut a number of eggs passing from the 32-cell into the 42-cell stage, I have never seen spindles directed radially when  $x$  and  $y$  were present. I hoped by tracing the cells into the next division to determine whether  $x$  and  $y$  were pushed in; if these two cells in that place were wanting, they might be accounted for in this way. I did not succeed, however, because the descendants of  $G^a, H^a$ , and  $G^b, H^b$  take such different positions that it is impossible to be sure of the following generation. For instance, in one egg the arrangement was

$$\begin{array}{c} G^a H^a \\ G^{a'} H^{a'} \\ G^{b'} G^b H^b H^b \end{array}$$

as shown in Fig. 23. In another

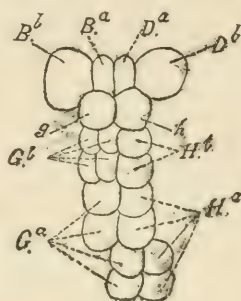
$$\begin{array}{c} G^a \\ G^{a'} H^a H^{a'} \\ G^{b'} G^b H^b H^{b'} \end{array}$$

while in a third it was

$$\begin{array}{c} G^a H^a \\ G^{a'} H^{a'} \\ G^b H^b \\ G^{b'} H^{b'} \end{array}$$



so that in the next stage I was not able to decide from their position whether  $g^a$ ,  $h^a$  had divided again or whether certain of the other cells had divided. I did find, however, one egg with about sixty-four cells, in which the two cells next to  $b^b-b^a$ ,  $d^a-d^b$  were as large as  $g$  and  $h$  are before dividing. The arrangement of the other cells is diagrammatically sketched in the cut. It was necessary to make a diagram, because all the cells could not be seen in the same field of view. In this one egg I did not find the two cells in the center. I cut sections to see if I could find spindles going in, but the critical section was completely broken. I know there were no cells in the interior for the reason that when they are present they can always be distinguished in optical section in the whole cleared egg.



After this time the two cells in the interior are found in various stages of disintegration. As late as the 112-cell stage, after the blastoderm has appeared on the surface of the egg, two deeply stained patches can still be seen in the interior. Shortly after this stage they disappear altogether, and no other cells are seen in the yolk until the egg is about forty-eight hours old (Fig. 39). Weismann and Ischikawa ('87) have described three secondary polar bodies in *Bythotrephes longimanus*, which are carried into the interior of the egg during the early segmentation stages. These polar bodies subsequently disintegrate, though as late as the 32-cell stage remnants of them could still be detected in the axial space between the blastomeres. Dr. Mead ('95) also has found that the polar bodies of *Amphitrite* are taken into the axial cells, where they are absorbed. These results of Weismann and Ischikawa and of Mead led me to suppose that the two cells found in the interior of the *Microdeutopus* egg were polar bodies. I therefore made a careful study of the eggs before the 32-cell stage. Since I found no cells which had not been derived from one of the two blastomeres of the 2-cell stage, I conclude that the cells in the interior cannot be polar bodies. In the literature which

I have seen I have found nothing to which I might compare these two cells. Dr. McMurrich, however, found two cells in the interior of an advanced isopod egg; but as they were seen only once, and no traces of disintegrating nuclei were found in the later stages, Dr. McMurrich supposed it to be an abnormality. It may be, however, as Dr. McMurrich suggested to me, that the two cells found in the interior of the isopod egg are comparable to those which I have described for *Microdeutopus*. Dr. Conklin has kindly permitted me to state that he, too, finds that two cells (the tip cells in one arm of the cross) in *Crepidula* are lost in the later stages. However, they are *not*, as I understand, absorbed, but thrown out. It would be interesting to know if these cells in *Crepidula* could be compared to those in *Microdeutopus*. Comparisons have been made between the amphipod and molluscs before (Ulianin '81), with how much right is still to be decided. But certainly the loss at an early stage of two blastomeres is very remarkable, and has as yet not been described for other forms.

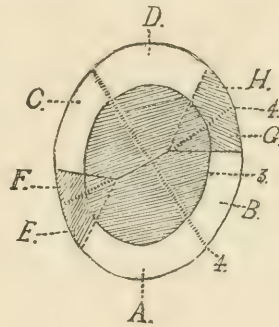
*44-cell stage.* — The blastomeres  $E^a$ ,  $F^a$  divide, giving rise to  $E^{a1}$ ,  $F^{a1}$  and  $E^{a2}$ ,  $F^{a2}$ . The cleavage is parallel to the planes which divided  $E$  and  $F$  before. Fig. 21 shows an egg passing from the 44-cell stage into the 46-cell stage. The blastomeres  $E^{a2}$  and  $F^{a2}$  are dividing for the last time before they rise to the surface of the egg.

*73-cell stage.* — Figs. 14–16 represent three views of an egg of seventy-three cells. In this egg  $E^{a1}$ ,  $F^{a1}$ ,  $E^{a2}$ ,  $F^{a2}$ ,  $E^b$ , and  $F^b$  have all been divided by vertical planes at right angles to the last, and the micromere  $f$  has also divided in the same way. The protoplasm in these cells has come up to the surface, and the nuclei have become very large and clear. This is the first appearance of the blastoderm. The sixteen large macromeres have divided into thirty-two by an equatorial cleavage.  $a^b-a^a$ ,  $c^a-c^b$ , and  $b^b-b^a$ ,  $d^a-d^b$  have not changed, and there are seventeen cells derived from the  $gh$  and  $GH$  groups. The descendants of  $A$  and  $C$  lie next to each other and border upon the  $EF$  group, while the  $B$  and  $D$  groups are forced apart by the  $GH$  group. Sections (Fig. 26) show the protoplasm quite near the surface at this time, the cell boundaries breaking down at their

inner ends, and the blastocoel becoming obliterated. The section shows one of the two cells which have wandered in. Its nucleus appears as a dark, homogeneous mass, showing signs of disintegration.

*106-cell stage.*—The thirty-two large cells all divide once more, by vertical cleavages, into sixty-four. I should state here that, although I have always spoken of the descendants of the four large macromeres of the 16-cell stage as large, I have done so merely to distinguish them from the descendants of the small macromeres. Somewhat before the 73-cell stage, owing to their more rapid cell division, the difference in size has disappeared, and in the living egg, as stated by Mles. Pereyaslawzewa ('88), Rossiiskaya ('88), and Wagner ('91), the macromeres and micromeres cannot be distinguished. In the cleared egg they are recognized by the different appearance of their nuclei and the smaller amount of protoplasm contained in the micromeres.

It will be recalled that in the 16-cell stage *A* and *C* were flattened against each other, while *B* and *D* were forced apart by *G* and *H*. Because of the obliquity of the second cleavage furrow and the flattening of the larger macromeres against each other, the group of small cells lies obliquely on the oval egg, as shown in the diagram. Since, as stated before, no change in the position of the cells takes place, their descendants have exactly the same position as the cells themselves originally had. So the *AB* group lies over the pole nearest the *EF* group, while the *CD* group lies over the opposite pole, and the *A* and *C* groups lie nearest each other and border the *EF* group. At this time the protoplasm of all the blastomeres rises to the surface (Fig. 17). The protoplasm of the macromeres becomes more concentrated, while that of the micromeres spreads over the yolk, thus making such a thin layer that only the dark nuclei remain visible (Fig. 18). Since the concentration of the



protoplasm of the macromeres is toward the *EF* group, the cells of the *AB* group, as can be seen by the diagram, would come to lie over the lower pole, whereas the cells of the *CD* group would be drawn away from the upper pole and lie on the side of the egg, and the whole ventral plate so formed would lie eccentrically over the oval egg. Figs. 17 and 18 show an egg at this stage. The eleven cells at *EF* are the descendants of the *EF* group, the twelfth cell of this group lying beneath the surface, as was shown by sections. The end cells of the *CD* group can be seen in Fig. 18 on the side of the egg, while the end cells of the *AB* group cover the lower pole. The cells of the *EF* group will form the head region and the dorsal organ. Bessels ('70) is inclined to believe that the dorsal organ arises exactly at the same point where the blastoderm first appears on the surface of the egg, but, according to my observations, it does not appear exactly at this point, but a little lower down, as the cells of the *EF* group, which are the first blastodermic cells to appear, spread during their growth. In sections of an egg at this stage cells are seen underneath the surface in the *EF* region, and I think these are the descendants of *ef*, because the sections of the egg represented in Fig. 17 show five cells beneath the surface in this region. One of these is, I think, the twelfth cell of the *EF* group, as only eleven were seen on the surface. It is only in this way that the *ef* group can be accounted for. It will be remembered that only four cells were derived from this group. Another reason which led me to this conclusion is that all the other micromere cells are overgrown as the embryo develops and come to lie in the lower layer.

#### *Summary.*

The first cleavage plane appears three hours after the deposition of the egg. The three succeeding divisions, vertical, equatorial, and vertical, occur at intervals of an hour each, and after this the large macromeres divide synchronously and regularly, a vertical cleavage alternating with an equatorial cleavage; but the micromeres no longer divide synchronously with the macromeres.



The *EF* group and the micromeres divide only vertically.

The ventral plate is formed by the descendants of the large macromeres and of the *EF* group, and has an oblique position upon the egg, owing to the obliquity of the second cleavage plane.

After the 42-cell stage two cells are found in the interior of the egg in different stages of disintegration.

#### *Formation of the Embryo.*

In the last section I have described the ventral plate as being formed by the macromeres on the lower pole of the egg. I am well aware that in this I stand alone, all previous writers describing the descendants of the micromeres as the first which rise to the surface. Further, they describe the embryo as forming over the micromere pole, and the macromeres as gradually added to the outer layer during the growth of the ventral plate over the egg. Della Valle, who studied *Microdeutopus* as a control observation, agrees in this point with what has heretofore been published. He describes the blastoderm as arising on the micromere pole in *Orchestia*, and makes no exception in the case of *Microdeutopus*. The results which Mlle. Wagner obtained for *Melita* agree most nearly with what I found for *Microdeutopus*. According to her account, when the cells emerge from the yolk they lie on the sides as well as on the oral pole of the egg, and later grow over the dorsal face. This corresponds almost exactly to what I have found. The figures 4, 5, and 6 of *Sunamphitoë*, by Mlle. Rossiiskaya, are almost identical to those I have for sections of eggs in the stage figured on Pl. XXVI, Fig. 17. Her figures show that the blastoderm probably arises in the same way as in *Microdeutopus*.

The *EF* group lies above the plane of the equator on the side of the micromeres, and the cells of this group are the first which appear on the surface to form the blastoderm. Previous investigators may have been led by this to suppose that the ventral plate forms on the micromere pole, but, having traced the development cell by cell on the stained and cleared egg, I



am convinced that *the ventral plate is formed from the descendants of the macromeres and over the macromere pole.*

The pole upon which the ventral plate is formed will become, as in all other Crustacea, the ventral side of the embryo. The cells after reaching the surface rapidly increase in number, and as the embryo grows backward over the egg it shifts its position, so that its long axis finally corresponds with the long axis of the egg. In Fig. 29 we can still see that one side of the ventral plate is a little nearer the posterior pole than the other side, the shifting at this time not having been completed. Fig. 35 shows an egg in which, for some reason, the shifting has not taken place, so that at this late stage the embryo is still oblique upon the egg. In Fig. 33, a stage which is a little later than that shown in Fig. 29, the ventral plate has grown almost over the posterior pole (Fig. 32), and the axis of the embryo lies parallel to the long axis of the egg.

The rotation of the embryo upon the egg has been described by Bergh ('93) for *Gammarus pulex*, in which the embryo lies at first parallel to the shorter axis of the egg, and rotates during development through an angle of 90°. Bergh said he could not explain why it arose in that position. Della Valle also figures the embryo as lying at first obliquely over the egg, then parallel to the short axis, and, lastly, parallel to the long axis. I could not make out, however, whether he supposed a rotation to take place, or whether he considered the short axis to be drawn out at the expense of the long axis. In *Microdeutopus* we have seen that the cause of the oblique position of the embryo upon the egg is due to the obliquity of the second cleavage plane.

In the stages represented in Figs. 33 and 34 the outlines of the cells are quite sharply marked off from the yolk. Their arrangement in definite lines is partly due to the fact that the blastodermic cells appear on the surface of the egg arranged in definite rows, on account of the regular cleavage of the two large macromeres. Yet this cause cannot hold good for the sides of the embryo, for in Fig. 28, the side view of an egg shown in Fig. 29, the spindles lie at all sorts of angles to each other, and the cells have at this time no definite arrangement,

although later they fall into line. In the isopods and in Mysis teloblasts give rise to regular rows of cells in the postnaupliar region. In *Microdeutopus*, however, I could find neither ectodermal nor mesodermal teloblasts. Bergh supposed that the regular arrangement of ectoderm cells, which is found in the amphipods, arose from some indistinguishable teloblasts, but I think this assumption is not necessary for *Microdeutopus*, since the regular arrangement is found also in the naupliar region (Figs. 27 and 31), where no teloblastic growth occurs.

The dark patches (*d*) in Figs. 28, 29, 32, and 33 represent cells below the surface, which, I think, have been overgrown by the ventral plate as it extends over the egg. In the isopods, as described by Dr. McMurrich, and in Mysis, as described by Bergh, the cells scattered over the dorsal pole are added to the ectodermal layer of the ventral plate as it grows over the egg, and with these facts in mind I searched carefully to see if this was the case with *Microdeutopus*. Further and careful examination only strengthened the view that in *Microdeutopus* the dorsal cells are overgrown and so form part of the lower layer. At a little later stage (Fig. 34) the head region has increased in extent, a few ectodermal cells are beginning to differentiate at the sides, and the edge of the ventral plate is even more sharply marked off from the yolk than in the preceding stages. In the next stage (Fig. 30) the patch of dark cells has become very characteristic in appearance. The dorsal organ (*d.o.*) has begun to differentiate, and the outline of the ventral plate is no longer distinct. It appears as though a layer of protoplasm had spread over the yolk, and through this layer the nuclei are found irregularly scattered. This appearance is due, I think, to the lack of definite cell walls, for I have never been able to distinguish them in *Microdeutopus*. In the earlier stages the cells seem to be "räumlich centriert," as Flemming expresses it; therefore, in eggs of stages represented in Figs. 29, 33, and 34, the cell boundaries can be distinguished, but in the later stages, where the cells become closely packed, cell outlines are lost. In eggs after the stage represented in Fig. 34, it seems as though the cells at the edge of the ventral plate have no longer the power to assume the spherical form, so making

the outline of the cell distinct, but their protoplasm spreads out over the surface of the yolk, as seen in section (Fig. 47, *ppl.*). As the cells in this region multiply, the protoplasm of one cell fuses with that of the surrounding cells (Fig. 47, *r.*), so that it appears as though a layer of protoplasm, through which the nuclei are scattered, was spreading over the surface of the egg, as shown in Fig. 30. The large cell (Fig. 30, *d'*) near the region of the differentiated cells lies below the two small nuclei which are imbedded in the protoplasmic layer. The *d'* cell is almost always seen in this position, even before the ventral plate has reached that point (Fig. 34). In this case, therefore, it is unmistakably one of the dorsal cells which has been overgrown.

As the embryo develops, the cells which are spreading dorsally arrange themselves in definite rows corresponding to the rows of cells of the ventral plate. During the second day the yolk is completely overgrown by the ventral plate, the edges of which meet at the dorsal organ. At this stage the dorsal organ has moved to the center of the dorsal pole of the egg, and is composed of large triangular cells, whose apices are at the surface of the egg; the antennules are clearly defined, and the appendages could be seen just beginning to form. Fig. 31 represents an egg of the second day. The appendages have appeared as a series of ridges on the ventral surface, gradually shading off dorsally, and it will be observed that the series extends over the posterior pole and dorsal surface of the egg, reaching almost to the dorsal organ (Fig. 36). The appendages are formed by the pinching off of from eight to ten parallel rows of cells, and while being pinched off each ridge includes some of the cells of the lower layer from which the mesoderm of the appendage develops. This mode of origin of the limbs and their musculature recalls what has been figured by Dr. Bumpus ('91) for *Homarus*.

The embryo now elongates, the area of growth being chiefly in the dorsal region, posterior to the dorsal organ, as shown in Figs. 36-43. By comparing these figures it may be seen that the distance between the dorsal organ and the last appendage increases in extent, in consequence of which a fold appears on

the ventral surface (Fig. 38, *abdf.*) in the region of the first abdominal appendage. Figs. 40-42 are three views of the same egg. At this stage the appendages no longer extend over the dorsal surface (*cf.* Figs. 36 and 40), although they still cover the lower pole of the egg (Fig. 41). The anlagen of the seven abdominal appendages are pushed inwards, and finally all lie in the abdominal fold (Fig. 43), whereas the space between the dorsal organ and the last appendage extends over the whole lower hemisphere of the egg.

The mode of formation of the abdominal fold in *Microdeutopus* differs widely from that in *Orchestia*, judging from the comparison of Figs. 36-43 for *Microdeutopus* with those of *Orchestia*, as figured by Della Valle ('93).

#### *Entoderm.*

The entoderm in *Microdeutopus* arises as a true invagination at the hind end of the embryo. During the second day, when the ventral plate has completely overgrown the egg, the cells immediately behind the dorsal organ invaginate. These cells migrate into the interior of the egg, and there arrange themselves to form the liver tubes and the greater part of the digestive tract. The entodermal invagination is shown in optical section in Fig. 37 (*en. inv.*), and Fig. 53 represents a transverse section passing through the center of the entodermal sac, while in Fig. 54, which represents a small portion of a sagittal section, the relative position of the entodermal invagination and the dorsal organ are shown. In this egg the cells of the dorsal organ have the characteristic bottle shape which is peculiar to them, but the ends of the cells still contain granular protoplasm; later they are filled with some clear, unstainable substance.

A section of an egg a little older (Fig. 55) shows the cells of the entodermal sac migrating into the interior as an irregular mass. These cells distribute themselves throughout the yolk area, the greater number, however, remaining in the region at which they entered. These are the first cells which appear in the center of the egg, excepting the two blastomeres



described above, which were absorbed. In eggs at about the stage represented in Fig. 33 I have seen cells in the anterior part of the head region which were apparently migrating off into the yolk area, as the one shown in Fig. 49, but these lay very near the surface, and in the stages shortly before the entodermal invagination had appeared cells were never seen in the yolk area. I conclude, therefore, that these were probably dorsal cells which were overgrown, and had not taken their final position at the time when the egg was killed. Cells which appear to be similar to these were described by Dr. Pereyaslawzewa ('88), who considers them to be entoderm cells derived from the ventral plate and migrating into the yolk area. I do not believe, however, that in *Microdeutopus* they take any part in the formation of the entoderm.

Ulianin ('81) finds a number of cells scattered through the yolk area which he supposes are derived from the dorsal organ, as at first they are found in that region. He leaves the origin of these cells an open question, however, as he was not able to secure a complete series of embryos, and, therefore, could not trace them to their source. The whole entoderm, according to Ulianin, is derived from these cells, and the lower layer cells of the ventral plate form mesoderm only. Dr. Pereyaslawzewa ('88) believes that the entoderm is derived from two sources, from the ventral plate and from the dorsal invagination, which she considers to be the dorsal organ, as the following passage states: "À mesure que l'organe dorsal se développe, l'ectoderme avoisinant s'épaissit visiblement et garde pour longtemps cette configuration, vu qu'il ne détache aucun organe nouveau. Ce rôle passif qui lui est propre, me permet de le comparer à la plaque dorsal chez les Insectes. La dissemblance consiste en ce que chez ces derniers la formation de la plaque précède celle du tube, tandis que chez les Crustacés nous remarquons le contraire. D'après les recherches de M. Korotneff sur le développement de *Gryllotalpa* les cellules qui dérivent en grande nombre de la plaque dorsal s'introduisent dans les masses nutritives et après les avoir élaboré de manière à les préparer pour l'assimilation, qui aura lieu dans les cellules de l'intestine, elles se détruisent complètement.



Il est indubitable que chez les Gammarus et de plus chez les Orchesties l'organ dorsal, ainsi que l'ectoderme avoisinant, détachent de cellules; leur nombre n'est pas grand, elles sont tout à fait libérées et s'enfoncent dans le vitellus nutritif. Or, tandis que les cellules en question se logent dans les masses vitellines, les cellules entodermiques, d'une parfaite ressemblance avec les premières, sont aussi en voie d'y chevaucher; leur résidence simultanée dans le vitellus ne nous permet d'affirmer aucunement que les cellules issues de la plaque dorsale s'atrophient; aucune de mes préparations ne le prouve pas."

I think the plaque of cells from which, Dr. Pereyaslawzewa writes, no organ is derived is the dorsal organ, and what she calls the dorsal organ is the entodermal invagination. At a later stage a cavity is found in the dorsal organ in *Microdeutopus* which agrees, in this respect, with what Korotneff found in *Gryllotalpa*. The characteristic appearance of the dorsal organ cells led me to recognize the plaque of cells as the dorsal organ. Dr. Pereyaslawzewa, it would seem, occupies the middle ground between Ulianin ('81), who supposes the entoderm is derived from the dorsal organ alone, and Bergh, who derives all the entoderm from the ventral plate. Bergh does not hold that the entoderm is formed at many points on the ventral plate, as Dr. Pereyaslawzewa holds, to judge from her figures, but he supposes that "vielmehr entsteht dasselbe durch Einwucherung von Blastodermzellen an einer bestimmten Stelle die also dem Blastoporus entsprechen dürfte." In his figures Bergh marks the cells in the second layer of the ventral plate, in the naupliar region, *en*. My own results agree with those of Ulianin, who believes that *all* the cells in the second layer of the ventral plate give rise to mesoderm, and that *only* the cells carried in by the dorsal invagination form the entoderm. It would seem, then, that as far as the entoderm is concerned the amphipod agrees most nearly with what Bobretzsky has found to be the case in *Palaemon*, according to the statement of Heider.

*Mesoderm.*

At the time when the entodermal invagination takes place the mesoderm is completely laid down and the appendages have begun to pinch off, each ridge containing mesoderm cells, as described above and figured in Figs. 36, 37, and 55.

To judge from what takes place in the great majority of other Crustacea heretofore described, we should naturally expect to find the mesoderm arising from the region where the anterior lip of the entodermal invagination will be formed. In *Microdeutopus*, then, the extreme posterior end of the ventral plate would be the region where we should look for a proliferation of mesoderm cells, since that is the region of the entodermal invagination. However, the posterior end of the ventral plate is exactly the region where the smallest number of lower layer cells are found; in fact, except for the few dorsal cells which were overgrown, that region is composed of only one cellular layer, whereas the head region at the time is composed of two or even three layers, and patches of cells are found irregularly scattered under the ventral plate, decreasing in size and number as they approach the posterior end.

When the protoplasm rises to the surface of the egg to form the blastoderm the four cells of the *ef* group were found under the cells of the *EF* group. The dorsal cells, which are the descendants of the remaining micromeres, are overgrown by the ventral plate, and, subsequently, form part of the lower layer of cells. In Fig. 18 only the dark nuclei of the dorsal cells can be seen, their protoplasm being spread over a large surface, making such a thin layer that it cannot be distinguished. As the ventral plate grows over the egg the protoplasm of the dorsal cells concentrates around their nuclei, and the cells present the stellate appearance shown in Figs. 27 and 34. Fig. 46 shows a section of the head region of an embryo at the stage shown in Fig. 30. A large cell (*d*) is seen just below the edge of the ventral plate. The beginning of the yolk area is shown at *Y*, and this area was greater in extent in the next section, showing that the cell above the large stellate one is at the edge of the ventral plate. Fig. 48 also shows

a large, clear nucleus (*d*) below the outer edge of the ventral plate, while in Fig. 47 the protoplasm (*ppl.*) of a cell also at the edge of the ventral plate has spread out over the surface of the yolk for some distance, and a large stellate cell (*d*) is seen beneath it. I do not think that these cells arise by division from the ventral plate, as Della Valle ('93) supposes. I draw this conclusion because there is no cell above the one shown in Fig. 36 from which it could divide, and especially because these cells at this stage are very different from those of the ventral plate, and their nuclei are similar to those of the dorsal cells. It may be that they are analogous to the vitellophags of the isopods, which arise from the *D* cell, described by Dr. McMurrich. The dorsal cells of *Microdeutopus* and the vitellophags of *Jaera* resemble each other in three points: in that they appear during the segmentation stage, in that their nuclei have a characteristic appearance, and in that they are overgrown by the ventral plate. The vitellophags in *Jaera* also give rise to mesoderm. The dorsal cells in *Microdeutopus*, however, take no part in the digestion of the yolk. After the ventral plate has completely inclosed the egg the dorsal cells have lost their characteristic appearance, and they cannot be distinguished from the other cells of the second layer.

I have still to add that, to judge from the appearance of embryos like the ones shown in Figs. 29, 32, and 33, the cells of the *GH* group also are overgrown by the descendants of the *AB* and *CD* groups.

In the head region of the embryo, in the stages shown in Figs. 29 and 33, numerous cells are found in the lower layer,<sup>1</sup> whereas only a few large stellate cells (overgrown dorsal cells) are found under the ventral plate in the postnaupliar region. At a little later stage (Fig. 30) there are more cells in the lower layer of the postnaupliar region than could be accounted for by the division of the stellate cells. They are found in patches irregularly scattered throughout the region of the ventral plate. As I had a very complete series of embryos, I do not believe that

<sup>1</sup> The mesoderm cells are not represented in Figs. 27 and 31, because they were so numerous at these stages that they could not be well represented, and because the regular arrangement of the ectoderm would have been obscured.

a plug of mesoderm cells arises at any one point, and that this stage had escaped my notice, but rather that in *Microdeutopus* the mesoderm is formed at many points in the ventral plate. I have a section of a cell dividing obliquely inwards in the anterior end of the head region, and Fig. 51 shows another case in which there are two cells in the aster stage. The equatorial plate of each spindle makes an acute angle with the tangent to the surface of the egg at that point. Had *A* divided, the greater part of one daughter cell would have lain in the second layer. In Fig. 52 we see a cell (*m*) which may have been derived from a cell like *h*, or it may be one of the cells of the outer layer drawn under the surface; from the appearance of the nucleus I am rather inclined to the latter view. I never have seen spindles whose axes were parallel to the radius of the egg in cells at the surface, although I have seen radially directed spindles in the second layer (Fig. 50). Cells which appear as though they were drawing in or had arisen by oblique division were found at any point on the ventral plate. I therefore conclude that part of the mesoderm in *Microdeutopus* is derived from the ventral plate.

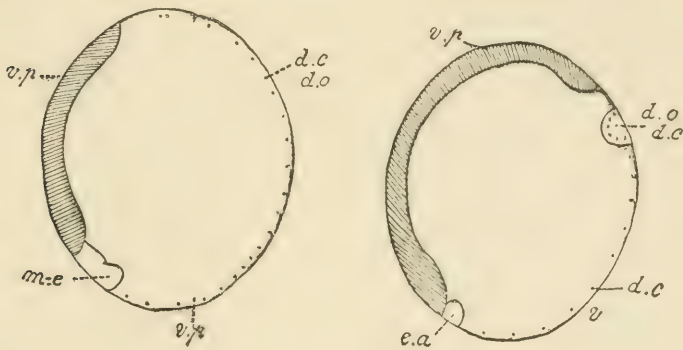
When the dorsal pole of the egg has been completely overgrown by the ventral plate the ventral portion of the blastoderm, posterior to the head region, is composed of two layers of cells. With the digestion of the yolk by the entoderm cells, which begins during the third day, all the cells of the embryo rapidly increase in number. Cell boundaries are entirely lost at this stage, the protoplasm of the cells fusing and making it impossible to distinguish where one layer ends and the other begins. Since the nuclei of both ectoderm and mesoderm appear exactly alike, I could not tell whether the ectoderm was only one layer deep and the mesoderm many layered, or whether the ectoderm consisted of more than one layer. Towards the end of the third day numerous spindles are found in all the layers making any angle with the tangent to the surface. In sections of the fourth day (Figs. 59-64) the muscle cells show their characteristic striations.

Bergh ('93), in his paper on *Gammarus*, suggests that the cells of the muscle plates are derived from teloblasts, as he



found to be the case in *Mysis*. In *Microdeutopus* I find no evidence of a teloblastic growth. The mesoderm cells seen in optical section in the stained and cleared egg are irregularly scattered under the ventral plate.

Morphologically, the isopods and amphipods are very closely allied, and it is, therefore, all the more interesting to note how similar are their modes of development. If we compare a diagram of *Jaera*, after the germ layers have been laid down, with



one of *Microdeutopus*, somewhat before the entodermal invagination has taken place, the similarity will at once become apparent (see diagrams).

Heider ('91), in writing upon the formation of the isopod embryo, states that the dorsal cells, as the embryo develops, become pushed together, and these cells later undergo degeneration. On page 352 he adds: "Es ist die Möglichkeit, die wir oben andeuteten nicht ausgeschlossen, dass in dem Dorsalorgan bloss die Involutionsform des Nahrungsdotter bedeckenden Blastodermtheils vorliegt. Die Involution wurde sich dann bei den Amphipoden-Typus durch Einstülpung, bei den *Oniscus*-Typus durch Amputation einleiten." If such is the case, then in both forms we have a ventral plate covering the ventral pole, at the posterior end of which the entoderm arises. Behind the entoderm lie the vitellophags or the dorsal cells in the amphipods, the similarity between these has been pointed out, and beyond the vitellophags the dorsal cells of the isopods or the dorsal organ of the amphipods, according to Heider.



The great difference lies in the origin of the mesoderm, and more work will have to be done upon other amphipods before this can be decided. It, however, is a great question to my mind if the dorsal cells in the isopods and the dorsal organ of the amphipods can be homologized. Heider himself merely suggests the possibility, as his conclusion was drawn solely from the work of Bobretzsky ('74).

If we compare embryos of the later stages we find that in both cases the embryo is folded dorsally (see diagram and Figs.



36, 37). In the amphipod, however, when the cells which will form the dorsum of the animal develop, the least resistance to the pressure exerted through their growth seems to be on the ventral pole, and, therefore, the embryo folds over ventrally (Figs. 38 and 42), whereas in the isopods the least resistance is in the dorsal region. Might not the different mode of folding in the two cases be due to the fact that by the time the cells which will form the dorsum of the animal develop the ventral pole in the isopods is further differentiated than is the case in the amphipod, and, therefore, offers greater resistance to the pressure exerted upon it by the growing region?

#### *Formation of the Liver Tubes and the Intestine.*

As has been described above, the entoderm arises as a true invagination. After the closure of the blastopore the cells migrate into the yolk area as an irregular mass, and by amoeboid motion migrate to both sides of the body in much the

same way that Bobretzsky ('74) describes for *Oniscus*. These become the liver tubes. Fig. 51 represents a section of an egg which is in about the stage shown in Fig. 38. The section passes somewhat obliquely through the region marked *a-a*. In the region of the dorsal organ the cells are arranging themselves to form a tube on the left side of the body. The sections (Figs. 57, 58) of a stage between Figs. 38 and 42 pass through the lines *a-a* and *b-b*, represented in Fig. 42. In the section lying nearest the dorsal organ (Fig. 58), where the greater mass of invaginated cells was found, the tubes are almost complete, and a mass of cells is still seen in the center of the egg. Only the dorsal walls are formed in the section nearer the anterior end, although in this case there are as many cells found in the part of a tube as there are in the whole tube of section (Fig. 58). This, however, is not always the case. The tubes are always complete in the region of the dorsal organ before they are complete in any other region of the body. Although no cell walls could be seen, the entoderm cells can be distinguished readily from those of the ectoderm and mesoderm by their nuclei, and in one egg there was a little yolk space between the cells of the body wall and the liver tubes. In other eggs the tubes have been seen in various stages of formation; for instance, in one section, where there was a break in the wall of the tube, like the one shown on the right-hand side of Fig. 58, three cells were lying in the gap, but they had not sent out protoplasmic prolongations at the time to complete the tube. In a section of an egg somewhat older (Pl. XXVII, Fig. 45), the liver tubes are complete in the region of the dorsal organ, and a few cells are found in the center of the yolk mass. Only in one instance have I seen an entoderm cell dividing. It was in the ring of the liver tube, and the plane of cleavage was parallel to the radius of the tube. I feel confident, however, that the cells must increase in number by division, because, as I shall show, most of the digestive tract is formed from entoderm cells.

By this time the elongation and consequent folding of the embryo, described above, has been completed. The area between the dorsal organ and the last appendage now extends

over the whole dorsal region of the abdomen. The blastopore, evidently, is carried over the posterior pole of the egg, and finally lies at or near the extreme tip of the abdomen (*cf.* Figs. 36-43). In consequence, the proctodaeum, which invaginates just posterior to the last appendage, forms either in the blastoporic area or just anterior to it, as is the case in the decapods. In the decapods, however, the stomodaeal and proctodaeal invaginations form the greater part of the intestine, while in the amphipods both stomodaeum and proctodaeum are very short. Figs. 59-64 represent sections of an embryo of the fourth day cut parallel to the line  $x-x$  (Pl. XXVII, Fig. 44). Owing to the folding of the embryo, the sections pass through stomodaeum and proctodaeum, cutting them transversely, while the dorsal sections cut the digestive tube horizontally. In Fig. 59 we see both stomodaeum and proctodaeum. The cells are closely packed and columnar. In the next section (Fig. 60) the stomodaeum is still seen as a round tube, but the proctodaeum has broken through. Another section showed that the stomodaeum has also broken through. On examining Fig. 61 it will be seen that the part of the digestive tract immediately adjoining the stomodaeum and proctodaeum is not formed at this time. Six large nuclei are seen bordering the yolk area in the thoracic region, whereas only three long, spindle-shaped cells could be seen bordering the yolk of the abdominal region. Two sections beyond this (Fig. 62) show the anterior ends of the liver tubes. The cells have large vacuoles, and inclose the whole yolk area. In the next section (Fig. 63) the liver tubes are pushed somewhat apart, and the irregular mass of cells between them represents the digestive tract just beginning to form in this region. In Fig. 64, where the section passes through the dorsal organ, the digestive tract is completely formed of large, vacuolated cells, with large, clear nuclei looking exactly like those of the liver tubes. Another section shows the digestive tract shading off into the large yolk areas of the thoracic and abdominal regions. All the yolk in these sections is inclosed in the liver tubes and the digestive tract. I have never seen any outside of them being digested by special vitellophags as Dr. Pereyaslawzewa ('88)

figures in Gammarus, or as Dr. McMurrich ('95) describes for the isopods.

We have seen how the liver tubes formed first in the region of the dorsal organ, where the greater mass of the invaginated cells lay; now we find the digestive tract completely formed in that region, whereas, anteriorly and posteriorly, it has not begun to form. The cells also present exactly the same appearance as those of the liver tubes; therefore, I conclude that the whole digestive tract, except the short anterior and posterior portions, is formed from the invaginated cells. If cells from the ventral plate or from the stomodaeum or proctodaeum were carried in to form the digestive tract, then I should expect to see the ends formed before the central portion.



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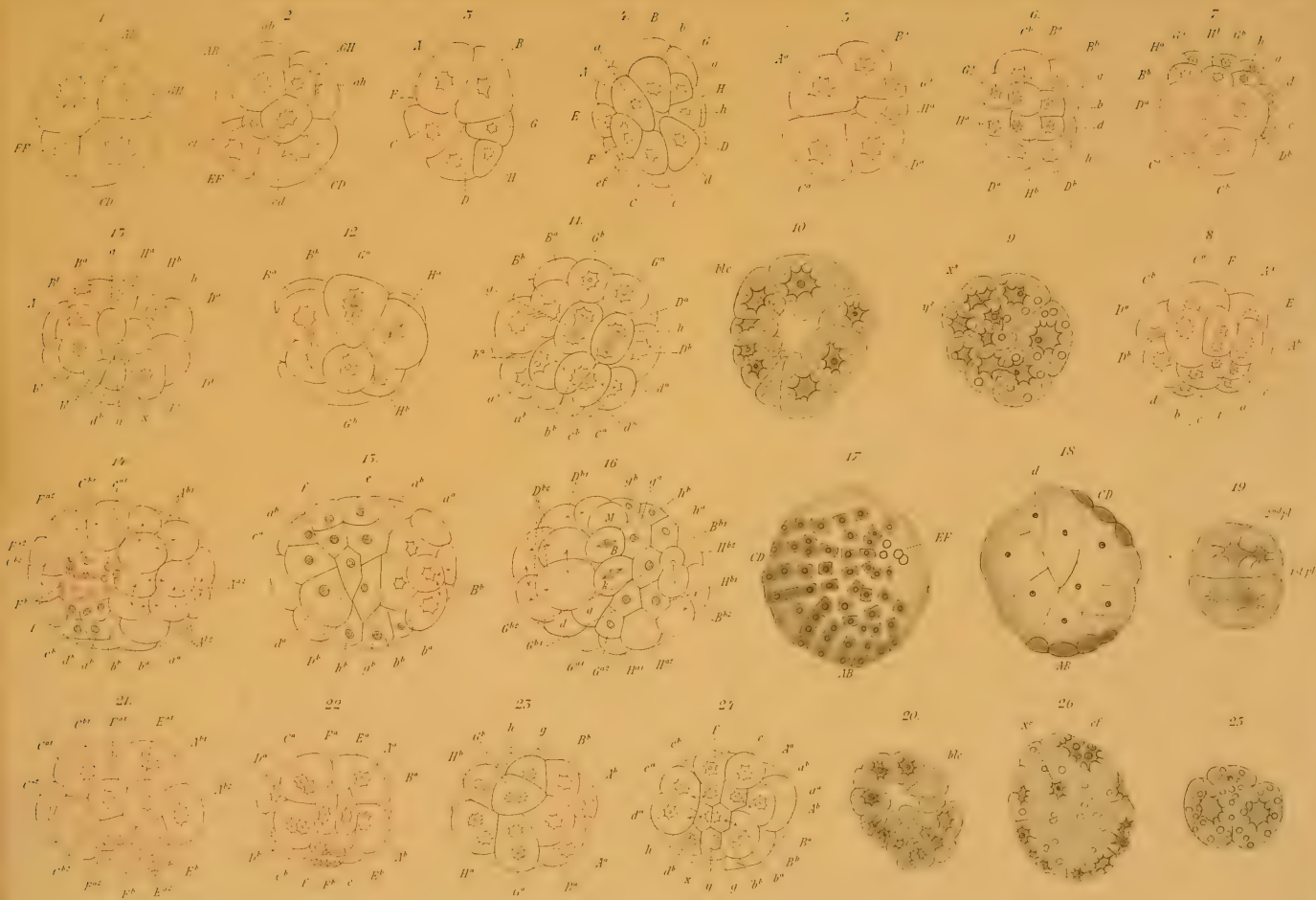
<i>abd.</i>	abdomen.	<i>en.inv.</i>	entodermal invagination.
<i>abd.f.</i>	abdominal fold.	<i>L.</i>	liver tube.
<i>an.</i>	antennae.	<i>l.a.</i>	last appendage.
<i>ap.</i>	appendage.	<i>m.</i>	mesoderm.
<i>blc.</i>	blastocoel.	<i>ppl.</i>	protoplasm of cells at the edge of the ventral plate.
<i>c.th.</i>	cephalothorax.	<i>pr.</i>	proctodaeum.
<i>d.</i>	dorsal cell.	<i>st.</i>	stomodaeum.
<i>D.O.</i>	dorsal organ.	<i>x and y.</i>	degenerating cells.
<i>ec.</i>	ectoderm.	<i>Y.</i>	yolk area.
<i>en.</i>	entoderm.		

## EXPLANATION OF PLATE XXVI.

- FIG. 1. 4-cell stage.  
FIG. 2. 8-cell stage from micromere pole.  
FIG. 3. 16-cell stage from macromere pole.  
FIG. 4. 16-cell stage from micromere pole.  
FIG. 5. 22-24-cell stage from macromere pole.  
FIG. 6. 22-24-cell stage looking down upon *GH* cells.  
FIG. 7. 22-24-cell stage seen from *CD* side.  
FIG. 8. 22-24-cell stage looking down upon *EF* cells.  
FIG. 9. Section of an egg of forty-five cells passing through the two blastomeres found in the blastocoel.  
FIG. 10. Section of the same egg shown in Fig. 7 passing through the blastocoel.  
FIG. 11. 28-40-cell stage looking down upon the *gh* cells.  
FIG. 12. 28-40-cell stage looking down upon the *GH* cells.  
FIG. 13. 40-cell stage showing *x* and *y* cells.  
FIG. 14. 73-cell stage looking down upon the *EF* group.  
FIG. 15. 73-cell stage from micromere pole.  
FIG. 16. 73-cell stage looking down upon the *GH* group.  
FIG. 17. 102-cell stage from the macromere or ventral pole after the blastoderm has risen to the surface of the egg.  
FIG. 18. Micromere or dorsal pole of the egg shown in Fig. 17.  
FIG. 19. Optical section of an egg passing from the 2 into the 4-cell stage.  
FIG. 20. Section of an egg of forty-three cells, showing one cell with the spindle at right angles to the surface of the egg.  
FIG. 21. 44-46-cell stage looking down on *EF* group.  
FIG. 22. 30-42-cell stage looking down on *EF* group.  
FIG. 23. 30-42-cell stage looking down on *GH* group.  
FIG. 24. 30-42-cell stage seen from micromere pole.  
FIG. 25. Section of an egg of the 8-cell stage.  
FIG. 26. Section of an egg of 72-cell stage.













## EXPLANATION OF PLATE XXVII.

- FIG. 27. Dorsal view of an egg of about twenty-four hours.  
FIG. 28. Side view of the egg shown in Fig. 27.  
FIG. 29. Ventral view of the egg shown in Fig. 27.  
FIG. 30. Side view of an egg of about thirty-six hours.  
FIG. 31. Anterior pole of an egg of about thirty hours.  
FIG. 32. Posterior pole of the egg shown in Fig. 31.  
FIG. 33. Ventral view of the egg shown in Fig. 31.  
FIG. 34. Side view of an egg of a stage between those shown in Figs. 30 and 31.  
FIG. 35. Ventral view of an abnormal egg of about the same stage as that shown in Fig. 33.  
FIG. 36. Dorsal view of an egg of the second day.  
FIG. 37. Side view of the egg shown in Fig. 36.  
FIG. 38. Side view of an egg about the end of the second day.  
FIG. 39. Ventral view of the egg shown in Fig. 38.  
FIG. 40. Dorsal view of an egg of about the beginning of the third day.  
FIG. 41. Ventral view of the egg shown in Fig. 40.  
FIG. 42. Side view of the egg shown in Fig. 40.  
FIG. 43. Side view of an egg at the end of the third day.  
FIG. 44. Side view of an egg of the fourth day.  
FIG. 45. Section of an egg of a stage shown in Fig. 43.













## EXPLANATION OF PLATE XXVIII.

- FIG. 46. Transverse section of the head region of an embryo shown in Fig. 30.
- FIG. 47. Transverse section of the same egg about the middle of the ventral plate.
- FIG. 48. Transverse section of the same egg nearer the posterior pole.
- FIG. 49. Transverse section of the head region of an egg of the first day.
- FIG. 50. Transverse section of an egg of the first day.
- FIG. 51. Part of a section of an egg of the first day.
- FIG. 52. Part of a section of an egg of the first day.
- FIG. 53. Transverse section passing through the center of the entodermal invagination.
- FIG. 54. Small portion of a sagittal section, showing the relative position of the dorsal organ and the entodermal invagination.
- FIG. 55. Sagittal section of an egg of the second day.
- FIG. 56. Oblique section of an egg of the stage represented in Fig. 38 passing through the region *a-a*.
- FIG. 57. Section of an egg of a stage between 38-42 passing through *a-a*, Fig. 42.
- FIG. 58. Section of the same egg passing through the line *b-b*.
- FIG. 59. Section of an egg of the stage shown in Fig. 44, passing through the stomodaeum and proctodaeum and cut parallel to the line *x-x*, Fig. 44.
- FIG. 60. Another section of the egg shown in Fig. 59.
- FIG. 61. A section dorsal to the one shown in Fig. 60.
- FIG. 62. Thoracic portion of the second section beyond the one shown in Fig. 61.
- FIG. 63. Thoracic portion of the next section to the one shown in Fig. 62.
- FIG. 64. Thoracic section beyond the section shown in Fig. 63.











THE REGENERATION OF THE NERVOUS SYSTEM  
OF PLANARIA TORVA AND THE ANATOMY  
OF THE NERVOUS SYSTEM OF DOUBLE-  
HEADED FORMS.

SIMON FLEXNER.

DURING the summer of 1895, through the courtesy of Prof. C. O. Whitman, I enjoyed the pleasure of spending two months in the Marine Biological Station at Woods Holl. At the suggestion of Dr. Jacques Loeb, who extended to me the privileges of the Physiological Laboratory, I began the study which forms the subject of this paper. The following year I obtained additional material, which was sent to me at Baltimore, where the investigation was continued and brought to its present state of completion. My thanks are due to both these gentlemen for many acts of kindness, and to the latter for much valuable advice.

The drawings which accompany this paper I owe to the kindness of Dr. Alice Hamilton.

The researches of Loeb<sup>1</sup> upon heteromorphosis have shown that it is possible, through the use of several different procedures, to bring about a substitution of an organ of one physiological value for that of another, and even to cause to be developed in one part organs which normally belong to widely removed localities. The light which the observations have thrown on the underlying forces and the manner of the formation of organs is considerable ; the study of the histological details of the process of organ-building in such cases has been little pursued.

That heteromorphosis can be produced experimentally in the planarian has been proven by Van Duyne,<sup>2</sup> whose studies also

<sup>1</sup> Untersuchungen zur physiologischen Morphologie der Thiere, Würzburg, 1891 and 1892.

<sup>2</sup> Ueber Heteromorphose bei Planarien, *Archiv f. die ges. Physiologie*, LXIV, 1896, 569.

indicate the ease and variety of the development in these forms of duplicate parts. A knowledge of the histology of the regenerative phenomena in the nervous system is of interest, therefore, not alone in itself for its bearing on the question of the growth of highly specialized organs, but as affording a basis for a closer study of the phenomena of heteromorphosis in these animals.

The methods employed in this study consisted in (*A*) decapitation of the worms ; (*B*) decapitation to which was added an incision in the longitudinal axis, passing through the entire thickness of the animal, and extending about one third of its length ; (*C*) complete longitudinal division with and without decapitation. At different periods in the process of regeneration the worms were killed with  $\text{HgCl}_2$ , formalin 5%, alcohol or osmic acid (Flemming's and Hermann's solutions), hardened and sectioned in paraffin. A number of staining agents were employed.

The decapitation was performed on the extended animals, the precaution having been taken to remove the entire cephalic extremity and contained nervous ganglia.

When partial longitudinal incision was also carried out, the object of which procedure was to produce double-headed forms, it was found necessary, in order to prevent healing, to separate the incised halves every twelve hours or oftener for the first two or three days.

After complete longitudinal division, each half immediately becomes rolled up in the form of a spiral, which gradually unwinds as regeneration proceeds.

The rapidity of the regeneration of new and duplicate parts varies in several ways. The shortest periods were noted where a single extremity was replaced (head or tail); a longer period is required for the completion of two parts of the same sort (double heads); and the longest are observed in the regeneration of the longitudinally divided halves.

Temperature also plays an important part. At the quite uniform and moderately low temperature of Woods Holl, the growth takes place far less rapidly than at the much more elevated temperatures which prevail in Baltimore during the



months of July and August. As this study was begun at Woods Holl in 1895 during the corresponding months and completed upon material forwarded from Woods Holl to Baltimore in the summer of 1896, this comparison was easily possible.

The especial purpose of this inquiry was to ascertain the manner in which the nervous (cephalic) ganglia and nervous cord were regenerated under the several conditions mentioned, and also what the new relations were which arose in artificially produced monsters. A few words regarding the anatomy of the normal parts may properly precede a detailed account of our study.

The nervous system of the planarian with which I have worked consists of a cephalic and a trunk portion (Fig. 1). The cephalic extremity consists of two bulbous enlargements, lying near the lateral edges of the animal, on a line with the eye-spots. These are connected by a commissure which is somewhat smaller in dimensions than the ganglia, although it does not differ in structure from these parts. Passing outwards towards the caudal extremity are two cords, one proceeding from each ganglion, which run in a parallel direction quite to the end of the animal. These cords are connected by semicircular commissural processes which come off at somewhat irregular intervals. From the cephalic extremity, as well as from the nervous cords, nerves pass outwards to the periphery of the body.

There is no essential difference in the microscopical structure of the ganglionic masses and the nervous cords. Each consists of a bundle of very fine non-medullated nerve fibers in close proximity to nerve cells. These cells — ganglionic cells — are more numerous in the cephalic ganglia. But cells similar in type and appearance exist in the cords, and may even be found, although much more sparsely, in the nerves themselves. In the ganglia they exist within the bundles of nerve fibers, grouped in the center, scattered in the periphery but most thickly placed in the outermost zone. In the cords they are found quite regularly disposed among the fibers.

The process of regeneration was studied in all stages, beginning twelve hours after decapitation, until the complete restora-

tion was effected. At the end of the first twelve hours and about equally at the conclusion of eighteen hours, active cell proliferation in the divided end is going on. The evidences for this are found in the rich mitosis encountered as well as in the accumulation of small, immature cells at the injured extremity. The most active division is found in the tissues immediately adjacent to the epidermal elements close to the superficial epithelial cells and to a far less extent in the cells at a distance (Fig. 2). The surface epithelial epidermal elements show no evidence whatever of mitosis; while close to the site of operation degenerative changes, consisting of fragmentation of cell nuclei (karyorhexis), are to be made out. Although especial attention was directed to the ganglion cells in the nerve cords at this time, mitotic figures were never demonstrated in them.

The new cells accumulate about the cut end and quickly cover over the defect. At the end of twelve hours they have pushed forward to the extremity, have become continuous with the intact epidermal cells, and have already formed a covering for the denuded structures (Fig. 3). Whether an embryonic form of epidermis or not, considerable masses of multinucleated protoplasm (syncytium) form the outermost layer of new cells.

At the end of twenty-four hours cell division in the regenerating end is quite over. Long and painstaking search is required to discover a single karyokinetic figure. But the cellular accumulation is now considerable. The new cells not only cover in the defect and are evident to the naked eye as a projecting white point, but they surround and inclose the divided nerve cords. The changes noted at the end of forty-eight hours consist in a somewhat more orderly arrangement of these cells about the cord and the invasion by them to a small extent of the bundle of fibrils themselves (Fig. 4).

The completed ganglion is found at about the sixtieth hour (Woods Holl temperature), although at this time the cephalic extremity does not appear to have attained its maximum size and the regenerated epidermis is still unpigmented. It may happen that in the ganglia at this time there is an undue rich-

ness of ganglionic cells (Fig. 5), while in specimens four days old no difference from the normal can be detected (Fig. 6).

More detailed changes are as follows: At the end of twenty-four hours the new cells have increased greatly and have quite filled up the cut surface, covering over the alimentary tube, etc. The cells have increased in size; they are oval or oblong in shape, and exhibit vesicular nuclei. They inclose the cut ends of the nervous cords and lie often in parallel rows, the long axis of the cells being placed in the horizontal plane. The most considerable group of cells is at the cut extremity and *fibers are already visible* between these cells. It cannot be positively stated that these cells have not migrated inwards among the old fibers and that these latter are the objects seen between them. But on the other hand, certain cells which lie to either side and just above the cords show similar fibers. It would be difficult to account for the presence of these upon the supposition that they were pre-existent; it appears more probable that they are newly formed. This conclusion is impressed upon one the more as these fibers are less distinctly linear and wavy than those in the cords themselves. Many of them are indeed short and granular, and some may not be fibers at all, but perhaps a sort of granular intercellular substance. This material is, however, not present in other places between cells; and it seems limited to the region of the old nerve cords. It is conceivable that the granules are derived from young and immature fibers altered by the fixing and hardening process.

These cells present the characters of neuroblasts, and are doubtless destined to compose the reproduced cephalic nervous structures (Fig. 7).

The histology of the growth of the nervous ganglia in the double-headed is not distinct from that of the simple forms. The gross anatomical result is, however, quite different. Although ganglia, two in number, connected by a commissure and closely resembling the normal structures, come to be developed, each is in association, not with a pair of nerve cords, but with a single cord (Fig. 8). The eye-spots bear about the same relation to the ganglia as they do in animals with single heads.

The regeneration of longitudinally divided animals goes on with far less rapidity ; finally, however, the result is quite as perfect as in the other instances. What takes place at once after section is, as already stated, a curling of the halves, the unraveling of which may require two weeks or even longer. As the restoration of the removed parts precedes this unwinding the degree of regeneration may be inferred from the extent of the return to the normal form.

The manner of the production of an anatomically complete individual is of much interest. Confining our description to the nervous system it is evident that the new segment could be produced in the growing half in one of two ways. Either a new and independent formation of the removed parts which become united with the remnant of the old system takes place, or the segment of the old system furnishes the starting-point of the regenerating half.

The cellular changes following median division are similar to those which succeed to simple decapitation, in so far as the exposed segment of the nervous mechanism becomes surrounded with cells which are gradually differentiated into ganglion cells and fibers. This increase in cells which, undergoing successive differentiation into neurones, gradually extends the nervous elements into the growing half, gives rise in succession to the cephalic ganglion, including commissure and nerve cord, thus projecting, as it were, the old system into the new part. There is, then, not a formation *de novo* of the removed segment, but a continuous outgrowth from the intact half (Fig. 9); the new cells do not arise by division of the old nerve (ganglion) cells, but come from other sources.

The phenomena attending regeneration of the central nervous system have been studied in both vertebrates and invertebrates, and with somewhat varying results by different observers. Most investigators agree in having found that in vertebrates the capacity for reproduction of removed or injured parts is very slight or even *nil*.<sup>1</sup> While Coën, von Kahlden, Sanarelli, and Friedmann found that defects in the cerebrum and cerebellum were replaced by connective tissue, and

<sup>1</sup> See Barfurth, *Regeneration*, Merkel-Bonnet's *Ergebnisse*, i, 1891, 132.



Schiefferdecker failed to observe regeneration in the spinal cord of mammals, Danilewski<sup>1</sup> claims to have demonstrated in a frog in which one cerebral hemisphere had been removed nine months before, a newly formed cerebral mass containing young nerve cells.

To the proof furnished by Mingazzini<sup>2</sup> that in tunicates complete restitution of the brain, etc., takes place, may be added the observations of Friedlander on the manner of the reproduction of the central nervous organs in certain worms. According to this author, the restoration takes place by an outgrowth from the remaining normal portion. It is, however, not clear from Friedlander's<sup>3</sup> study just what the origin of the nerve cells may have been, nor what is the nature and origin of the leucocytes which give rise to a compact, cellular new tissue.

My studies of the manner of regeneration of the central nervous organs of the planarian are capable of a different interpretation. There is no evidence of an outgrowth proceeding from the intact and non-degenerated nerve cords, nor is there any indication of a proliferation of the nerve cells existing among their component fibrils. The new organ results from a new growth of cells which originate independently of the old nervous system and in close proximity to the epidermal covering. They are not the fully formed and completely differentiated epidermal cells; for in these there were never found active changes which could be regarded as regenerative in character. It seems much more probable — although complete proof cannot at present be adduced — that the actively dividing elements are of the nature of *Ersatz* cells, which, while destined to give rise to the surface epithelium, are still capable of being transformed into neuroblasts.

A simple outgrowth from the several nervous cords can be excluded. For, besides the fibers which compose the ganglia,

<sup>1</sup> See Barfurth, *Regeneration*, Merkel-Bonnet's *Ergebnisse*, iii, 1893, 171.

<sup>2</sup> Ueber die Regeneration bei den Tunicaten, *Bol. della Societa di Naturaliste in Napoli*, 1891, ser. I, vol. v, p. 76. Ref. Merkel-Bonnet, *Ergebnisse*, i, 1891, 122.

<sup>3</sup> Ueber die Regeneration herausgeschnittener Teile des Centralnervensystems von Regenwürmern, *Zeitschr. f. wissenschaftl. Zoologie*, lx, 1895, 249.



a more considerable and important constituent is furnished by the nerve cells. First appearing as indifferent cells, entirely without orderly arrangement, they quickly undergo changes through which they become disposed in a regular manner, agreeing in position with the original cells which make up the parts, and after suffering some further alterations in form and staining capacity, may even be seen to develop fibers, which it is fair to assume are nervous in character. There occurs, also, at first an over-production of new cells, a surplus which in the end has disappeared. It would appear as if the new cells somewhat distant from the nerve trunks do not develop fibers, nor do they assume so orderly an arrangement; and, moreover, they are the individuals which are finally lost.

If this view of the regeneration of the nervous ganglia is correct, it must be admitted that a metaplasia of cells destined to become a simple external protective organ into the most highly differentiated structures of the body is, in these forms, possible. Barfurth<sup>1</sup> has pointed out the similarity in the regeneration of the epithelium of the skin and that of the central nervous organs of amphibian larvae, and attributes this to their common origin from the ectoderm. This conception, moreover, contains less that is startling in view of the observations of Wolff and Erik Müller<sup>2</sup> on the regeneration of the crystalline lens in tritons from the epithelium of the iris.

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<sup>1</sup> Regeneration, Merkel-Bonnet's Ergebnisse, i, 1891, 132.

<sup>2</sup> *Archiv f. mikroskop. Anatomie d. Entwicklungsgeschichte*, xlvii, 1896, 23.



## EXPLANATION OF PLATE XXVIII A.

FIG. 1. Semi-diagrammatic representation of the nervous system of *Planaria torva*. *A.A.* nervous ganglia; *B.B.* nervous cords. Ganglion cells indicated in gray.

FIG. 2. Karyokinesis in cells adjacent to the site of injury. The greatest activity in and increase of cells is near the ectodermal covering. Two fields are shown, *A.* near, *B.* somewhat removed from the injury. 12 to 18 hours after decapitation.

FIG. 3. New cells at the site of injury. Pushing forward of the cells to cover the defect, the new elements becoming continuous with the ectodermal cells. Large, thin, protoplasmic masses (syncytium) cover the extreme edge. *A.A.* cells pushing forward to epidermis; *B.B.* syncytium; *C.C.* masses of newly formed cells. 12 hours after decapitation.

FIG. 4. A nerve cord (*A.*) surrounded by new cells. The thickest group covers the cut end, and individual cells of the same type may be seen pushing their way among the fibers. Indistinct fibrillae appear between the cells above the cord. 48 hours after decapitation.

FIG. 5. Completion of the ganglion. Increased richness of cells on each side of the cord and normal radial arrangement around the bulbous end. Differentiated nerve cells show a lighter tone than the remainder. After 60 hours.

FIG. 6. Completely regenerated nervous system. *A.A.* ganglia; *B.* commissure; *C.C.* nervous cords. After 4 days.

FIG. 7. Showing neuroblasts next to and beyond the sectioned nerve cord. Only the cells in close proximity to the original cord exhibit processes.

FIG. 8. Complete double-headed form. *A.A.* nerve cords; *B.B.* ganglia. The new ganglia on each side are united by commissures, and to each pair a single cord is attached. Eye-spots shown.

FIG. 9. Almost completely regenerated longitudinally divided half. *A.A.* ganglia; *B.B.* cords. The cord *B.<sup>1</sup>* not quite perfect. After 13 days.







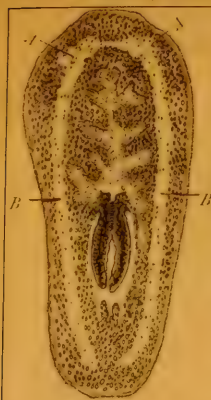


Fig. 1.

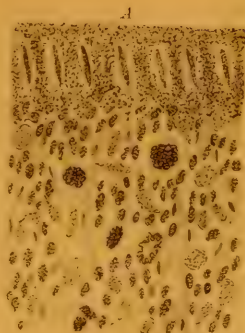


Fig. 2.

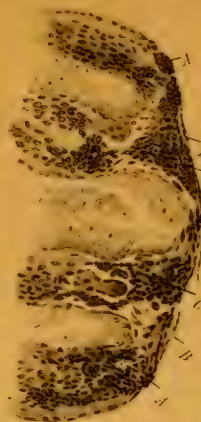
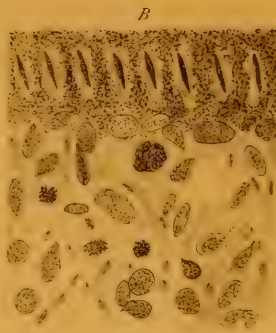


Fig. 3.

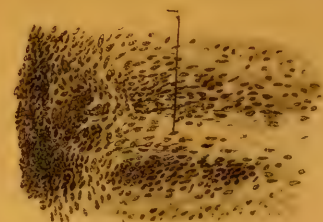


Fig. 4.

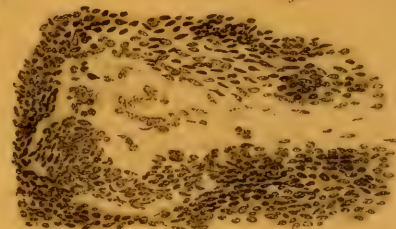


Fig. 5.

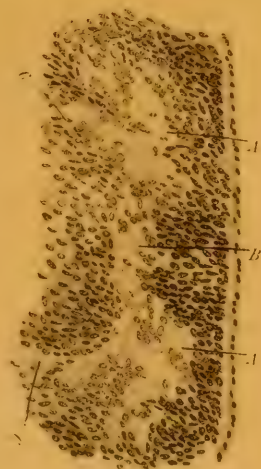


Fig. 6.



Fig. 8.

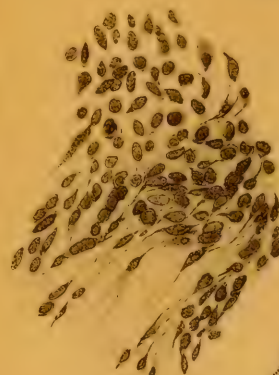


Fig. 7.



Fig. 9.



## DEVELOPMENT OF THE VENTRAL ABDOMINAL WALLS IN MAN.<sup>1</sup>

FRANKLIN P. MALL.

AFTER the neural plate is formed within the amniotic cavity, the tail end of the embryo remains attached to the chorion by means of the allantoic stalk (Bauchstiel). The amnion surrounds the embryo only on its dorsal side, as has been shown recently by the study of a number of young human embryos. The ventral side of the embryo, however, hangs free in the coelomic cavity, as the body walls have not been formed by the extension of the amnion. The extension of the amnion proceeds hand in hand with the flexion of the embryo. It first extends over the face of the embryo, then tucks under the tail end, and at the same time it encroaches from both sides of the body. In covering the head it first crosses the mouth and then the heart, this movement being accelerated by the growth of the amnion over the head end of the body from left to right.<sup>2</sup> While this is taking place, the stem of the umbilical vesicle elongates to produce the general condition, as represented in embryos four weeks old. Soon, however, the amnion covers the whole allantoic stalk, including within its folds the stem of the umbilical vesicle, thus forming the true umbilical cord. At this stage the intestine begins to enter the cord.

After we have reached the stage in which the umbilical cord is formed we find that in transverse sections of human embryos, as well as in embryos of other mammals of the same stage, the ventral abdominal walls are composed wholly of a membrane of connective tissue, without any ribs, muscles, or blood vessels as they are found in the adult.

<sup>1</sup> The present paper is to be considered a continuation of two papers published recently; one upon the development of the human coelom (*Journ. of Morph.*, vol. xii), and the other upon the development of the human intestine (His's *Archiv*, Supplement Band, 1897).

<sup>2</sup> Mall, *Journ. of Morph.*, vol. xii, p. 430, Figs. 27, 28.

It is now generally believed, and to a great extent proved, that the ribs and muscles wander into the ventral walls of the embryo from the sclerosomes and myomeres on the dorsal side of the embryo. It is easy to demonstrate the growth of the ribs from the dorsal to the ventral side of the embryo, but it is not so easy to demonstrate the growth of many muscles from the muscle plates to their final position in mammalian embryos. With the growth of the ribs and the wandering of the muscles from the myotomes into the ventral wall of the embryo we have associated with them their nerves and blood vessels, which ultimately form the intercostal nerves and blood vessels respectively. The segmental arteries fuse at their tips early in their development immediately below the rectus abdominis to form the internal mammary and deep epigastric arteries. This chain of anastomosis is formed in a manner with that seen in the formation of the vertebral artery, as shown by His, Froriep, and Hochstetter. The primitive internal mammary and deep epigastric arteries, together with the rectus, at first lie immediately below the mammary line in pigs' embryos, and all three, artery, muscle, and line, wander together towards the ventral middle line of the body. No doubt the same process takes place in the human embryo, but the mammary line lacks prominence here and cannot be used as a landmark.

*As the segmental nerves appear, each is immediately connected with its corresponding myotome, and all of the muscles arising from a myotome are always innervated by branches of the nerve which originally belonged to it.*

This generalization is difficult to prove in all of its details, but all of the work in embryology, as well as a tabulation of our knowledge of motor nerve distribution, indicates that it must be true. The simpler muscles of the back, as well as the intercostal, are innervated by their corresponding nerves. The muscles of the eye, of mastication, and of the face can be correspondingly grouped. The trapezius, latissimus dorsi, serratus anticus, and rectus abdominis wander great distances and carry with them their original nerves. Moreover, it would be practically impossible to explain why each muscle has its proper nerve supply, which is so evenly distributed in it, were



not this original relation between muscle and nerve retained. With this view we have no grave mechanical difficulties to overcome in conceiving how it is possible for the simple peripheral nervous system of the embryo to be evolved into that of the adult. It is simply that each segmental nerve grows to its myotome, or corresponding group of tissue or branchial arch, and with the further growth of muscles from myotomes the original nervous connections are retained. The required free growth of a nerve is then extremely short, as the myotomes receive them as soon as they leave the spinal cord, and then as the different muscles or parts of muscles arise from each myotome the nerve bundles split to correspond. The primitive muscle, then, is from its earliest appearance connected with the cord, and, as its muscle fibers appear and increase in number, this first connection guides the new nerve fibers to their destination.

The studies in comparative anatomy by Gegenbaur<sup>1</sup> and by Huxley<sup>2</sup> all lead to the same general conclusion that nerve and muscle are associated with each other in their earliest stages of development. This idea has been the foundation for a number of studies in comparative anatomy, and the more it is investigated the stronger the foundation becomes, as may be seen in the critical review by Kollmann.<sup>3</sup> In a discussion of Testut's "Anomalies musculaires chez l'Homme," Gegenbaur<sup>4</sup> shows clearly that the study of variations of muscles would have great meaning if their nerve supply were included, and that all the reports of muscle anomalies for a period of more than one hundred years cannot be used at present, due to this omission. The great value of the comparative study of the muscles in connection with their nerve supply has recently been shown again by Ruge.<sup>5</sup> His study shows that comparison of muscles can be made satisfactorily only when their nerve supply is included.

All this indicates that the history of a muscle is indicated by its nerve, and that in studying the development of a muscle our

<sup>1</sup> Gegenbaur, Grundriss d. vergleich. Anatomie.

<sup>2</sup> Huxley, Comparative Anatomy of Vertebrates.

<sup>3</sup> Kollmann, *His's Archiv*, 1891, p. 76.

<sup>4</sup> Gegenbaur, *Morph. Jahrb.*, Bd. x, p. 331.

<sup>5</sup> Ruge, *Morph. Jahrb.*, Bd. xix, p. 376.



main guide is its nerve. This has been plainly indicated recently by Nussbaum,<sup>1</sup> and in my studies in embryology and anatomy I cannot find a single instance to contradict this idea.

The same generalization, no doubt, applies also to the sensory nerves, which are distributed in a segmental way to the integument. Their first distribution would then be to the regions immediately over the spinal ganglia, and as the skin shifts in its development it carries with it its original nervous supply.

That the nerves cannot be distributed to any great extent without some guide is further demonstrated by all experiments on regeneration, which show that they have great powers of growth if they are guided in their distribution by a canal or by the connective tissue sheath of some degenerated nerve.

Although it is impossible to prove at present that all of the skeletal muscles arise from muscle plates or their corresponding coelomic diverticula into the branchial arches, the generalization that they do arise from the mesoderm segments is based upon sufficient observation to make it highly probable. In sharks the coelom extends into the branchial arches and into the myotomes; in man the cavity in the muscle plates is never connected with the coelomic cavity, while in the branchial arches no such cavity exists. Yet we do not hesitate to assert that the condition found in sharks is the primitive, while that in man is due to secondary changes. Of course the problem is more difficult when the fate of the plates is to be studied. In the sharks, where they are definite, it is easy to show that all of the skeletal muscles of the body arise from them, while in man it is extremely difficult to show from what myotome any given muscle arises. The first difficulty encountered is that in the head (with the exception of in the occipital region) there is no grouping of the mesoderm in the branchial arches, while in the extremities the myotomes lose their sharp outline and appear to blend with the surrounding mesenchym. This fact has been sufficiently emphasized by Paterson<sup>2</sup> a number of years ago, and recently has been used by Fischel<sup>3</sup> and by Har-

<sup>1</sup> Nussbaum, *Verhandl. d. Anat. Ges.*, 1894-1896.

<sup>2</sup> Paterson, *Quar. Journ. of Micro. Sci.*, N.S., vol. xxviii, p. 109.

<sup>3</sup> Fischel, *Morph. Jahrb.*, Bd. xxiii, p. 544.

rison<sup>1</sup> in combating the notion that all muscles arise directly from the myotomes. Harrison studied the development of the fin muscles in the trout, and finds that, with the exception of those in the pectoral fin, they arise directly through buds from the myotomes. In this one fin the muscle bud is small, and is so closely blended with the mesenchym that it is impossible to show whether or not the muscles of this fin arise from the muscle bud or mesenchym or from both. It appears to me that if all the body muscles of sharks and all of them in the trout arise directly from the myotomes, with the exception of those of the pectoral fin, it is better for the present to ascribe the muscles of this fin to the myotomes rather than to mesenchym, when mesenchym and buds are so completely blended.

Not only does Harrison<sup>2</sup> deny that all the skeletal muscles arise from the myotomes, but he also attempts to prove that the segments of a muscle, like the rectus, do not indicate that it has arisen from a corresponding number of muscle plates. He has shown that in teleosts the rectus is first split from the muscle plates as a band of tissue, and then undergoes secondary segmentation. After the muscle is laid down its front end remains connected with the myotomes until it has segmented, and hence these segments of the rectus correspond with the myotomes, while behind they do not. That the segments in the posterior end are formed after the muscle is separated from the myotomes indicates that there are mechanical causes which favor segmentation, but it is not proved that these segments do not correspond to the myotomes until it is shown that the nerve supply of the two are different. Even if it were shown that the succession of segments in the rectus did not correspond fully with the segmental nerves it would not prove anything special other than the history of this muscle. We must only recall the serratus anticus, which is composed of beautiful segments, all of which are of secondary formation. In its development the muscle wanders down from the neck, attaches itself to the scapula, and then successively to the ribs. During all this process of growth it retains its original nerve connec-

<sup>1</sup> Harrison, *Arch. f. mikr. Anat.*, Bd. xlvi, p. 500.

<sup>2</sup> Harrison, *Johns Hopkins University Circular*, No. 3, 1894.

tion, which shows its origin; that it is segmented is due to the ribs, a mechanical element.

While in the sharks it is comparatively easy to follow the development of the eye muscles with their respective nerve connections, it is impossible at present to follow them in higher animals. The same applies to the muscles arising from the hyoid arch and innervated by the seventh nerve. It is a remarkable fact, however, as emphasized by Rabl, that just those muscles which belong to the hyoid arch are innervated in man by the seventh nerve. Even where we can see no sharp line whatever in the embryo, there is one which has been indicated to us by the study of sharks. Rabl<sup>1</sup> states, after speaking of the muscles of mastication: "Die übrige Gesichtsmuskulatur dagegen wird vom Facialis innerviert und sie ist, wie Gegenbaur und Ruge auf vergleichend-anatomischem Wege gezeigt haben, aus einer Differenzierung des Platysma hervorgegangen; das Platysma selbst aber gehört genetisch dem Hyoidbogen an und wird daher, wie seine Differenzierungsprodukte, vom Facialis versorgt. Ebenso scharf sondern sich auch die beiden Innervationsgebiete in der Paukenhöhle. Hammer und Amboss entwickeln sich aus dem ersten, der Steigbügel, wie ich auseinandergesetzt habe, aus dem zweiten Kiemenbogen; der Muskel des Steigbügels vom Facialis innerviert. Der Musc. tensor tymp. gehört mit dem Tensor veli palatini genetisch und anatomisch zu einer Gruppe (Schwalbe); der Musc. stapedius bildet in ähnlicher Weise aller Wahrscheinlichkeit nach mit dem Stylohyoideus und hinteren Biventerbauch eine Gruppe."<sup>1</sup>

The short muscles of the trunk are innervated by single nerves, and in them the embryonic condition is retained. But in the case of the shifting of a muscle its nerve supply is not so simple as in case of the diaphragm. In this extreme example the muscle wanders down with the development of the septum

<sup>1</sup> Rabl, *Anat. Anz.*, Bd. ii, p. 226.

<sup>1</sup> There is a very extensive literature to show that the muscles of the limbs arise from the myotomes. The systematic work of Mollier (*Anat. Hefte*, No. viii) is one of the best of this group of papers. In my discussion above I have only brought forth some general objections to the views of Paterson and the much stronger and better arguments of Harrison.

transversum,<sup>1</sup> and its nerve supply indicates that the muscle must have arisen from the fourth or fifth cervical myotome or both. After the septum transversum has reached its final location, to form the diaphragm, muscles arising from the lower dorsal myotomes wander into its periphery and these are innervated by the lower intercostal nerves. In the same way the sterno-mastoid and trapezius grow over from the head to the shoulder, while the latissimus dorsi and serratus anticus grow from the shoulder to the ribs and pelvis. I have already spoken of the serratus anticus above, and wish to add a few words about the latissimus dorsi. It undoubtedly arises from the seventh and eighth cervical myotomes, is soon separated, and free to move. It attaches itself first to the humerus and then gradually spreads out over the back, attaching itself first to the vertebrae, then to the ribs, and finally to the ilium. As the growth takes place in this order the shoulder end is the older, and the pelvic end the younger, thus accounting for the nerve distribution as shown recently by Nussbaum.<sup>2</sup> In spreading, its fasciculi cross and encircle branches from the intercostal and lumbar nerves, thus making them perforate this muscle. Other muscles that shift, like the deltoid and pectoralis major, are likewise perforated but not innervated by nerves foreign to them.

Sometimes muscles which remain segmental cover metameres beyond their nervous supply. Quain's<sup>3</sup> *Anatomy*, in discussing the quadratus lumborum and complexus muscles, asserts that this indicates a reduction of the number of nerves, while from the above discussion it appears to me that it is not a reduction of nerves but an extension of the muscle beyond its original bounds.

Likewise a complete absence of an important muscle (trapezius), or a group of muscles (facial), is not necessarily to be accounted for by a degeneration of the nerve. Anything which arrests the development of the muscle in its very earliest stage will accomplish absence of the nerve, while a nerve destroyed at so early a stage might be replaced by a neighboring nerve through anastomoses.

<sup>1</sup> Mall, *Journ. of Morph.*, vol. xii, p. 395, Figs. 30, 41.

<sup>2</sup> Nussbaum, *Verhandl. d. Anat. Ges.*, 1894, p. 179; 1895, p. 26; 1896, p. 64.

<sup>3</sup> Quain's *Anatomy*, vol. ii, p. 354.



One of the most complicating elements in the study of the development of a muscle is the formation of nerve plexuses. In those instances in which there is but little cross shifting in the movement of a muscle, its nerve remains single or nearly so. But when there is considerable cross shifting very early in the development we have the formation of a plexus. Furbringer<sup>1</sup> has shown from comparative anatomical studies that there is a plexus formation in the nerves supplying the limbs which shift in development, and that the plexus formation indicates that the limb is wandering. In the development of the extremities in man it has been clearly pointed out by the method of reconstruction that the arm bud makes a rapid excursion from the head backward in the early embryo.<sup>2</sup> In so doing it appears to drag the nerves together, thus forming a plexus. The upper intercostal nerves do not shift and, therefore, do not form a plexus, while the lower intercostal nerves passing to the anterior abdominal wall shift in their development, and thus favor the plexus formation.

In this shifting and mixing of nerves the portions of the muscle plates which they supply are also hopelessly mixed, and this I think accounts, in part at least, for the fact that the muscle buds are no longer defined in the arm and leg by buds. But after the muscles are fairly well outlined they move on to their destination without any more mixing of nerve fibers further than additions which may grow along the existing trunks from the spinal cord. The differentiation of the muscles in the extremities takes place after the plexus nearest the spinal cord is formed, thus permitting of a second group of nerve anastomoses in the extremity. This process in the forearm, for instance, accounts for the double nerve supply of some of the muscles in it.

Physiologists have studied the distribution of nerves in muscles much more carefully than anatomists, and we have to thank Mays<sup>3</sup> for very careful description of nerve plexuses in

<sup>1</sup> Furbringer, *Morph. Jahrb.*, Bd. v, p. 324.

<sup>2</sup> His, *Abhandl. d. k. s. Ges. d. Wiss.*, Bd. xiv, p. 341, Taf. II; and Mall, *Journ. of Morph.*, vol. v, p. 459, Pl. XXX.

<sup>3</sup> Mays, *Zeit. f. Biol.*, vol. xx, p. 449.



muscles. In a segmental muscle like the frog's rectus abdominis, irritation of the nerve supplying one segment not only makes this contract but the rest of the muscle also, showing that there must be an anastomosis between the nerves supplying the different segments.<sup>1</sup> Therefore, the irritation of the nerve passing to a single segment sets the whole muscle to contracting. The heart is affected in a similar way, for irritation of the minutest sympathetic twig has the same effect upon the heart beat as the irritation of its main trunk.<sup>2</sup> And the same is true regarding the influence of the splanchnic nerve upon the muscle walls of the vena portae.<sup>3</sup>

In addition to the physiological verification of these nerve plexuses we have ample confirmation of them by Nussbaum, and very recently again by von Bardeleben and Frohse<sup>4</sup> for a number of muscles in man. So every bundle of nerves which leaves the spinal cord to pass to a muscle has a chance to mix its fibers once, twice, or three times, as the case may be, before it reaches the muscle fibers, and a nerve stimulus may pass to a given muscle in a number of ways and from a number of sources.

The plexus within the muscle will, no doubt, prove to be a valuable object for investigation, but it does not concern us much when we consider the origin of a given muscle. I have given it for the sake of completion and have discussed the subject somewhat extensively in order to show a number of difficulties in my way, as well as my standpoint in the present problem.

*Development of the Rectus.* — In the earliest human embryos which have been studied it is observed that the allantoic stalk and the umbilical vesicle are located much nearer the head than in the adult. The relation of these organs to the body of the embryo is shown in the accompanying figure, which is taken from an embryo not over two weeks old.<sup>5</sup> It will be noticed

<sup>1</sup> Mays, Ueber die Nervatur d. Musculus rectus abdominis d. Frosches, Heidelberg, 1886.

<sup>2</sup> Johansson, *Archiv f. Physiologie*, 1897, p. 103.

<sup>3</sup> Mall, *Archiv f. Physiologie*, 1892, p. 423; and Johns Hopkins Hospital Reports, vol. i, p. 148.

<sup>4</sup> von Bardeleben and Frohse, *Verhandl. d. Anat. Ges.*, 1897, p. 38.

<sup>5</sup> The anatomy of this embryo is fully described by me in the *Journ. of Morph.*, vol. xii, p. 417, and in His's *Archiv*, Supplement Band, 1897.

from the figure that the allantoic stalk and the umbilical vesicle must shift their position through a distance of at least twelve segments in order to gain the position they will hold in older embryos. The possibility of this shifting is all easily understood when we consider how soft the tissues are at this time, and the lack of any framework to hold the different parts of the embryo together. Later on, when nerve bundles and skeleton appear, the shifting still takes place, but by no means to as great an extent, and its course is now marked by the arrangement of the nerves as well as other tissues. At this stage, however, the most fixed points appear to be the myotomes, and from them I have made all of my measurements. For reasons given in the earlier descriptions of this embryo I have ascribed three of the muscle plates to the occipital region, making the first dorsal myotome immediately over the neurenteric canal. In later stages each myotome lies immediately over a spinal ganglion, and in referring to the myotomes I give them the numbers corresponding to the spinal nerves. This causes less confusion than by numbering them from the auditory vesicle backward, for by so doing we do not convey any idea of adult anatomy.

In embryos slightly older than the one figured above, the spinal ganglia and vertebral column are outlined, thus aiding us easily to locate the position of any of the organs. With the descent of the heart the communication of the umbilical vesicle with the midgut is rapidly cut off, and the stems connecting them, together with the allantoic stalk are then encircled with the amnion. All these intermediate stages are sufficiently well shown in His's *Atlas*, and I need not describe them.

At the end of the fourth week the umbilical cord is fully formed, as is shown by a number of specimens in my possession, two of which are especially valuable because there is no doubt whatever as to their being normal. One is the specimen I have studied a great deal, which was obtained from a criminal abortion, and the other was gotten from a murdered woman and sent me by the coroner's physician of Chicago. The first specimen (No. II) is somewhat older than the second (No. LXXVI), and for that reason is more valuable.

Fig. 2 is a profile of this embryo<sup>1</sup> drawn from the specimen before it was cut, with the outlines of the myotomes of the body added from a reconstruction. It shows the relation of the cord to the rest of the body and the extent of the myotomes have grown towards it. In the body itself the myotomes have distinct buds which are growing into the membrana reuniens, and each of these is intimately associated with its nerve. Where buds from the myotomes enter the extremities, their ventral tips appear to be fully blended to correspond with the blending of the nerves which enter the limbs.

The extent of the growth of the myotome into the membrana reuniens is shown in Fig. 3. It shows the marked bud from the myotome. Earlier stages need not be given, and a later stage has already been described and pictured by Kollmann.

There is, however, a time when the buds from certain of the dorsal myotomes blend to form the rectus, and I have not been able to obtain a good specimen to demonstrate this. A stage somewhat older than one pictured by Kollmann<sup>2</sup> will no doubt give the rectus arising from the myotomes. A good specimen of this desired stage is not at my disposal.<sup>3</sup>

Fig. 4 is from a reconstruction of the body of embryo XLIII, to which has been added the head taken from a photograph of the embryo before it was cut into sections. In the drawing only the superficial layer of abdominal muscles is shown. The form and extent of the rectus, as well as its relation to the external oblique, is indicated. The transverse lines in the rectus correspond with the digitations in the oblique, and each of these digitations with its corresponding segment in the

<sup>1</sup> Mall, *Journ. of Morph.*, vol. v, p. 459. Reference Handbook of the Medical Sciences, New York, Supplement, pp. 184, 391, 875. *Journ. of Morph.*, vol. xii, p. 431. His's *Archiv*, Supplement Band, 1897.

<sup>2</sup> Kollmann, His's *Archiv*, 1891, Pl. III, Fig. 1.

<sup>3</sup> His's embryo Ko (*Abhandl. d. k. s. Ges. d. Wiss.*, Bd. xiv) and one described by Fraser (*Trans. Roy. Acad. Med.*, Ireland, vol. xi, Pl. VI, Fig. 1) represent the missing stage between embryos II and XLIII. Since writing the above I have obtained an excellent human embryo from an operation for tubal pregnancy. The specimen, No. CIX, was hardened immediately in graded alcohol, and measured 11 mm. Sections show that the ribs are just beginning, and between them the intercostal muscles are sharply outlined. Projecting from them there are spouts which at points appear to be blended to make the rectus abdominis.

rectus is innervated by a single intercostal nerve and, therefore, must be viewed as belonging to the same myotome:

The lateral position of the rectus, which has already been noted by Kölliker,<sup>1</sup> is also easily seen in transverse section, as shown in Fig. 5. Unfortunately, the stages between XLIII and II are missing, but the lateral position of the rectus does show that a finished rectus is formed at a very early date, and that it must still shift one-half the distance around the body in order to obtain its final position. The muscle is nearer the middle line at its upper end than at its lower, due no doubt to the more rapid growth of the ribs than of the pubis, to which it is now attached.

In a later stage, Fig. 6, we have the same condition of things as in No. XLIII, only more advanced. In this reconstruction it was impossible to make out definitely all the serration of the external oblique and the segments in the rectus, because the sections were transverse to the embryo, while in XLIII they were sagittal. The transverse sections (Figs. 7, 8) show the exact position of the rectus at its different levels.

The two recti reach the middle line above the umbilical cord about the eighth week, while the main bulk of the intestine is still in the cord. At this time the ventral abdominal walls above the umbilicus are completed, and their further growth is simply a matter of expansion. About the end of the ninth week the intestine is withdrawn from the cord, the opening soon becoming closed by an adhesion of the edges of the periphery of the communication of the coelom of the cord and the abdominal cavity. As soon as this is accomplished the stem of the cord is surrounded by a membrane about 5 mm. in diameter, which in turn is encircled by the two recti. It is very easy with a probe to reestablish the old communication, and in so doing the membrane again returns to its former position around the end of the cord, showing by this reverse process its origin. Very soon after this membrane is formed the recti invade it also, thus completing the abdominal walls at this point.

*Relation of the Segments of the Rectus to the Umbilicus.* — Judging by the illustrations in the various atlases and text-books

<sup>1</sup> Kölliker, Grundriss, 1884, p. 340.



on anatomy there is considerable difference of opinion regarding the positions of the transverse lines in the rectus abdominis muscle. No doubt this lack of agreement is due to the variations in the arrangement of the muscle segments, for we know that in some instances all of them, as indicated by their innervation, may be present, while on the other hand a number of them may be but slightly marked or even wholly missing. As a rule a number of well-marked cross striations are present, but their position and number is differently pictured by different authors. Two of the newer atlases express very well the two views which are usually entertained. In Toldt's<sup>1</sup> atlas a muscle segment is immediately opposite the umbilicus, while in Spalteholz's<sup>2</sup> atlas this muscle segment is wholly above the umbilicus, placing the lowest transverse line immediately opposite the umbilicus. With the exception of the insertion of the muscle, the illustration given by Spalteholz corresponds with that given by Quain, Henle, Testut, as well as by our more popular English text-books, Morris and Gray. In them the insertion, as pictured correctly by Spalteholz, is given only in a diagrammatic way. I have examined a great number of cadavers with special reference to this muscle and find that the great majority of specimens correspond in every detail with Spalteholz's illustration.

The muscle segment immediately above the umbilicus then is the rule, but whether it arises from the same myotome in all instances is not proved until it is shown that it is always innervated by the same nerve. This question I have investigated carefully in a number of cadavers and find that in nearly all instances the muscle segment immediately above the umbilicus is innervated by the ninth intercostal nerve. All atlases in which the exact innervation of the rectus is given confirm the above observation.<sup>3</sup> The skin immediately over this segment is also supplied by the ninth intercostal nerve.<sup>4</sup> This then gives us a very fixed point to work from in the further study of the

<sup>1</sup> Toldt, *Anat. Atlas*, Wien u. Leipzig, 1896.

<sup>2</sup> Spalteholz, *Hand-Atlas d. Anat. d. Muscle*, Leipzig, 1896.

<sup>3</sup> See in the magnificent *Atlas* of Hirschfeld and Lévillé, Pl. LIII. This figure is copied extensively; for instance, Quain, vol. iii, p. 309.

<sup>4</sup> Head, *Brain*, 1893, 1894.



walls of the abdomen, and from it we can make all of our counts. The segment above the ninth is usually the eighth, but occasionally the eighth and seventh combined, while the one lying upon the ribs belongs to the fifth and sixth, or sixth and seventh, or fifth, sixth, and seventh segments, as the case may be. The portion of the muscle below the umbilicus usually represents three segments, the tenth, eleventh, and twelfth, but occasionally a portion of the first lumbar may be included. It is of course understood that the numbers of the segments indicate from which myotomes they arose.

The comparative studies of Ruge<sup>1</sup> show that the number of segments in the rectus increase as we pass down the scale of mammals, and the relation of the umbilicus to the segments changes in proportion to the number. As the number of segments increases, the umbilicus is pushed more and more away from the head. In man it is nearest the head, but when there is a variation in its position it is in the form of a reversion. Ruge's Figs. 12, 13, and 14 illustrate this well, showing that the umbilicus may be below the tenth segment.

I have shown in an earlier communication that in the shifting of the viscera during development there is a fixed point opposite the first sacral segment, beyond which they do not pass. Everything descends towards this point, and those which go farther protrude into the umbilical cord. With the extreme shifting of the viscera there is a partial shifting of the abdominal walls, which seems to be partly controlled by the fixed point opposite the sacrum. This lower and more fundamental fixed point appears to be the meeting point of two forces, one the movement from the head towards the tail, and the other in the opposite direction. The point is well established in embryo XXII, and may control to a great extent the further shifting of the abdominal muscles. If there is any meaning to this fixed point opposite the sacrum, it will be necessary to find that it lies farther and farther away from the head as we pass down the scale of mammals.

After the ninth segment has become definitely located just above the umbilicus the portion of the muscle between the

<sup>1</sup> Ruge, *Morph. Jahrb.*, Bd. xix, p. 376.

umbilicus and pubis is relatively much shorter than it is in the adult. The distance between the end of the sternum and the umbilicus is twice the distance between the umbilicus and the pubis in embryos of about eight weeks, while in the adult these two measurements are equal.<sup>1</sup> Before the two pubic bones have met to form the symphysis, this difference is still greater. This narrowed area between the pubis and umbilicus in the early embryos may possibly account for the blending of the three muscle segments of the rectus in this region. The appearance in embryos XLIII and XXII, Figs. 4 and 6, also indicate this.

*Shifting of the Milk Line in Pigs' Embryos.*—The invasion of the membrana reuniens by the rectus is also indicated by the shifting of the milk line. O. Schultze<sup>2</sup> has shown that the mammary glands in pigs arise as a ridge of ectoderm on either side of the body immediately to the ventral side of the Wolffian ridge. In a short time the milk ridge breaks up into islands, each of which marks a later mammary gland.

Figs. 9, 11, and 13 show the position of the milk line in pigs' embryos, giving the relative positions of the line in the three different stages. Fresh specimens show clearly that the abdominal walls are extremely thin on the ventral side of the line, while on the dorsal side they are thicker and apparently finished. Sections of the three embryos pictured in Figs. 9, 11, and 13 are shown in Figs. 10, 12, and 14. The sections show that the line lies immediately over the rectus, and from this we can locate the position of the rectus in any embryo before it is cut. The wandering of the rectus is indicated then in pigs' embryo by the shifting of the milk line.

*Development of the Internal Mammary and Deep Epigastric Arteries.*—Before the intercostal vessels are formed there appears to be a circulation through the membrana reuniens, as is indicated to us by a figure given by Kölliker,<sup>3</sup> as well as by the description of His.<sup>4</sup> Kölliker pictures a cow's embryo with

<sup>1</sup> See also the figures given by Merkel, *Abhandl. d. k. Ges. d. Wiss.*, Göttingen, Bd. xl.

<sup>2</sup> O. Schultze, *Anat. Anz.*, Bd. vii, and *Verhandl. d. Phys.-med. Ges.*, Würzburg, Bd. xxvi.

<sup>3</sup> Kölliker, *Grundriss*, p. 103, Fig. 58.

<sup>4</sup> His, *Anat. Mensch. Embryonem*, Bd. iii, p. 206, also Fig. 130.

the whole membrana reuniens filled with a minute plexus of veins which radiate from the myotomes towards the umbilical cord, while His describes this same region in the human embryo as filled with branches of the umbilical vein which empty into the sinus reuniens above, and into the umbilical vein below. According to His's description they arise when the communication between the umbilical veins and the sinus reuniens is severed. Although the picture given by Kölliker does not correspond with His's description it does not contradict it, nor is it peculiar to the cow's embryo. I have in my collection a well-preserved human embryo (No. LXXVI), in which the membrana reuniens is filled with a plexus of veins much like that in the cow's embryo. The specimen was taken from the uterus seven hours after the death of the woman, and without opening the ovum was hardened in absolute alcohol. All the vessels down to the capillaries are filled with blood, thus making it an excellent specimen for the study of the blood vessels. It seems to represent a stage somewhat more advanced than the one pictured by Kölliker, as the plexus of veins does not cover the whole membrana reunieus. The ventral wall of the heart near the liver contains no vessels, while the membrana reuniens covering the upper end of the heart is filled with a plexus of vessels which communicate with the capillaries of the mandibular arch. There is an extensive plexus through the arm and lateral body walls which extends through the membrana reuniens covering the liver, and finally encircles the cord and communicates with the umbilical veins.

The specimen just described must be about 22 days old and, although I have six other good embryos between 14 and 28 days old, I find no such plexus in the membrana reuniens, although in all but one of them (14 days) the arm shows a rich plexus of capillaries filled with blood. In stages older than four weeks I find no blood vessels in the membrana reuniens with the exception of that portion encircling the cord where there is a rich network of veins. Although I have a number of excellent specimens of five and six weeks, the membrana reuniens over the heart and liver contains no blood vessels until it is invaded by the ventral plate, which is accompanied

by the development of the intercostal vessels. In pigs' embryos the extensive membrane is also free from veins, with the exception of the zone encircling the cord, which again has a venous plexus more marked, however, than in the human embryo.

It appears, then, that during the third week of development, while the umbilical veins still empty into the sinus reuniens, an extensive plexus is formed throughout the greater extent of the membrana reuniens, which receives blood from the aorta on its dorsal side, and empties it into the umbilical vein on its ventral side. As the umbilical vein changes its position to enter the liver, this circulation through the membrana reuniens is broken up as a much earlier circulation though the umbilical vesicle was broken up.<sup>1</sup>

The earliest collecting vein for the descending aorta is the omphalomesenteric vein; next it is the umbilical vein, and finally, when the abdominal walls are completed, it is the cardinal. This in turn is partly converted into the vena cava inferior.

The arterial system is already well outlined in embryo II. The aortic arches and segmental arteries are sufficiently well marked to number them. The vertebral artery is in process of development; it being formed by a union of a number of segmental arteries, as shown by His,<sup>2</sup> by Froriep,<sup>3</sup> and by Hochstetter.<sup>4</sup> The sixth cervical segmental artery gives rise to the subclavian artery. The lower cervical, all the dorsal and lumbar segmental arteries, are concerned in the development of the thoracic and abdominal walls.

Fig. 2 shows the general extent of the dorsal myotomes, while Fig. 15 gives the extent of the arteries. It shows a simple arrangement from the vertebral to the hypogastric artery. The lower lumbar arteries are not shown. A section of this embryo is shown in Fig. 3. It shows the relation of the segmental arteries to the myotome. The segmental arteries supply primarily the spinal cord and ganglia in two groups of

<sup>1</sup> In *Journ. of Morph.*, vol. xii, Fig. 16, it will be seen that the aorta sends a number of segmental arteries to the umbilical vesicle. In a short time these all disappear, only to repeat themselves over the body of the embryo. In turn this second set disappears as soon as the umbilical vein enters the liver.

<sup>2</sup> His, *Anat. Mensch. Embryone*, vol. iii, 1885.

<sup>3</sup> Froriep, His's *Archiv*, 1886, p. 69. <sup>4</sup> Hochstetter, *Morph. Jahrb.*, Bd. xvi.



branches, one near the middle line and one more lateral. A more ventral group of segmental arteries supplies the Wolffian body. The blood from all these groups of arteries appears to be collected by the cardinal veins; certainly that from the ventral and lateral group is.

The lateral group gives rise to the intercostal arteries by first supplying the myotomes, and, as these grow into the *membrana reuniens* by a process of budding, the vascular loop follows it. In so doing the loop is first on the dorsal side of the sympathetic and finally on its lateral side, thus making the sympathetic cord cross the intercostal arteries and veins on their ventral side, as is the case in the adult.

No sooner is the vascular loop extended to the lateral side of the sympathetic than it begins to anastomose with neighboring segmental loops, as single vessels near the subclavian and hypogastric arteries, and as a plexus midway between these two. This gives us at this early period a complete anastomosis from the subclavian to the femoral artery, as Fig. 16 shows. It remains only for this system to shift around towards the median line with the muscle, nerves, and ribs to form the condition of things as found in the adult.

Fig. 16 shows that the upper and lower segmental arteries of the series do not correspond with the same in the adult. Above the superior intercostal is missing, while below there is only a small fourth lumbar present, and it arises from the middle sacral. Ilio-lumbar and circumflex-iliac arteries are altogether wanting, and I should judge from the relation of the arteries in embryo XLIII that the arch formed by the ilio-lumbar and circumflex-iliac is of secondary origin and has nothing to do with the segmental arteries. That they form anastomoses with the lower lumbar arteries in the adult can be explained in other ways.

The hypogastric artery is present long before the segmental arteries are formed near its junction with the aorta, and on that account we can no more call the trunk of the common iliac artery segmental than we can apply the same term to the descending aorta. We can only locate its origin in the neighborhood of the fourth lumbar artery.



Hochstetter has settled definitely that the subclavian is a branch from the seventh cervical segmental artery.<sup>1</sup> Between the seventh cervical (if we number the arteries with the nerves) and the third dorsal we have three segmental arteries. In Fig. 15 the segmental arteries in the region are all shown to be simple with the exception of the seventh, which sends a marked branch into the arm. From this stage to the one pictured in Fig. 16 there is a marked jump, but in it we see the intermediate stage between Fig. 15 and the adult.

All of the arteries below the vertebral are destined to go behind the sympathetic, and it is only excluded on account of its direction. In embryo XLIII (Fig. 16) the eighth cervical segmental passes on the ventral side of the nerve, which shows conclusively that it must either be a new artery or a secondary connection between the eighth segmental and subclavian. Since the first and second intercostal arteries pass behind the sympathetic in this embryo and in front of it in the adult, we must accept Hochstetter's opinion that the superior intercostal is formed by secondary connections between the upper intercostals and the subclavian. If the old connection remains, it forms the *arteria aberrans*.

I stated above that the sympathetic lies in front of the subclavian, while in the adult it lies in great part behind it. Hochstetter explains this change of position by a wandering of the trunk of the artery through the group of embryonic nerve cells. In the early embryos the sympathetic system is as a group of sprouts from the segmental nerves which cross the segmental arteries, and the sympathetic cord is of secondary formation. This cord grows very rapidly during the fifth and sixth weeks, and makes a great mass of cells extending from the vagus ganglion to the adrenals, connecting all of the branches with the segmental nerves to make of them *rami communicantes*. This all goes on hand in hand with the descent of the heart into the thorax. At the same time the arm is rotating towards the ventral median line, and drags with it

<sup>1</sup> Hochstetter numbers the segmental arteries to correspond with the vertebrae above them. Throughout I give the arteries the numbers of their accompanying nerves. He states that the subclavian arises from the sixth segmental, this being what I call the seventh cervical segmental.

the subclavian artery. In so doing the subclavian is caught in the sympathetic cord in its earliest stage, while the greater portion of the cord is developed on the dorsal side of the artery. The portion of the sympathetic which from the first lies on the ventral side of the subclavian becomes the ansa subclavia.

The descent of the heart into the thorax on the inside with the descent of the arm over the clavicle on the outside causes great tension on the upper intercostal arteries, and favors the new formation of blood vessels in a more direct line. This is the reason why the main branch of superior intercostal is a secondary and direct artery from the subclavian.

*Résumé.* — The permanent abdominal walls, then, are formed by its various structures growing from the ventral plate into the membrana reuniens. The muscles grow as buds from the myotomes, and their original segmental nature is retained in great part by the intercostal and abdominal muscles. Each segment in shifting retains its original nervous connection, so that the origin of each segmental muscle is indicated by its nervous supply.

The segmental arteries soon unite at their tips, much after the fashion of the formation of the vertebral, to form the deep epigastric and internal mammary arteries. This arterial bridge is complete while it still lies on the lateral side of the body, and then obtains its definite position by shifting around to the ventral middle line of the body hand in hand with the ribs and rectus abdominis muscle.

In pigs' embryos the milk line lies immediately over the rectus and the arterial arch, and it shifts towards the middle line with them. Not only can this be determined by sections but also by observing live embryos. In the latter instance a cutaneous vessel is seen along the mammary, which belongs to the internal mammary and deep epigastric arteries. So the rectus, milk line, and the arterial arch with its cutaneous branches mark the border of the ventral plate as it invades the membrana reuniens. .

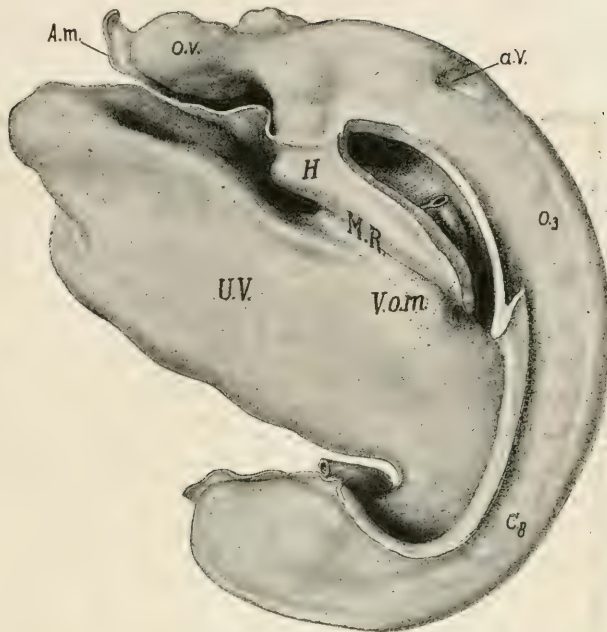


FIG. 1.—Profile view of embryo XII two weeks old. Enlarged 38 times. *A.m.*, amnion; *U.V.*, umbilical vesicle; *a.v.*, auditory vesicle; *o.v.*, optic vesicle; *O.3*, third occipital myotome; *C.8*, eighth cervical myotome; *H*, heart; *V.o.m.*, omphalomesenteric vein; *M.R.*, membrana reuniens.

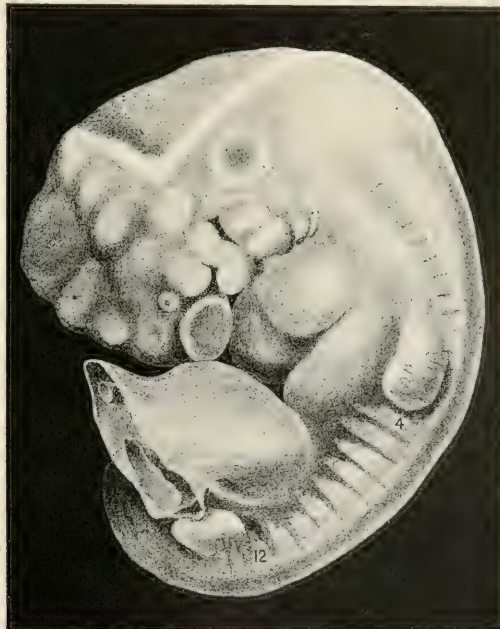


FIG. 2.—Profile view of embryo II four weeks old. Enlarged 10 times. The myotomes of the dorsal region have been added from the sections. *4*, *12*, fourth and twelfth dorsal myotomes.

*Broedel and Mall del.*





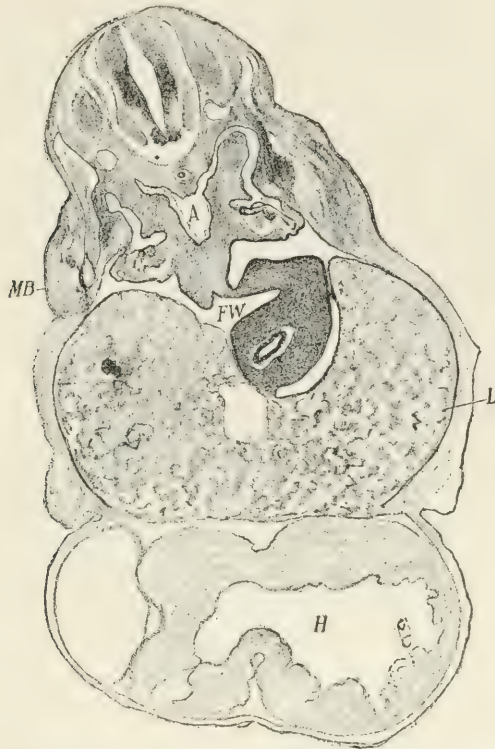


FIG. 3.—Section through the fourth dorsal myotome of embryo II. Enlarged 40 times. On one side the muscle bud, *M.B.*, is shown. *A.*, aorta; *FW.*, foramen of Winslow; *L.*, liver; *H.*, heart.

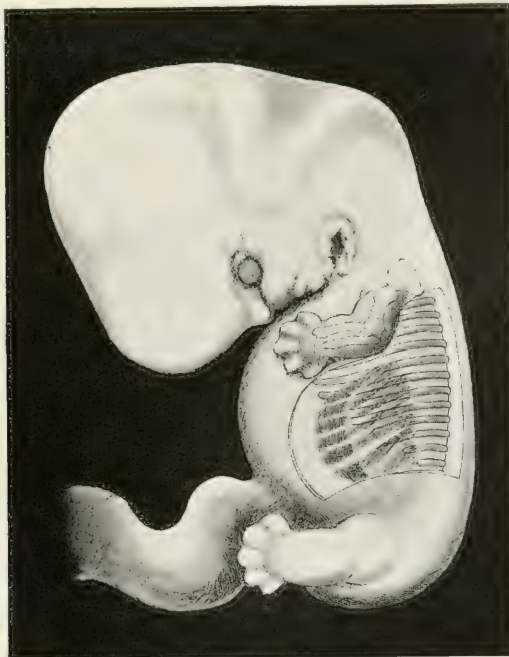


FIG. 4.—Embryo XLIII. Enlarged 5 times. The position of the rectus is well shown.  
*Schmidt del.*





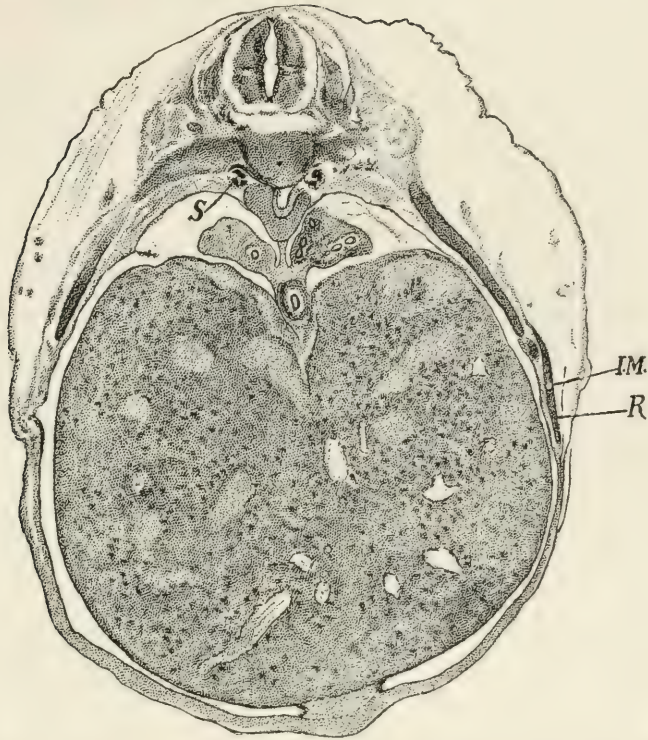


FIG. 5. — Transverse section through embryo LXXIV, being of the same stage as embryo XLIII. Enlarged 12 times. *R.*, rectus; *I.M.*, internal mammary artery.



*Schmidt del.* FIG. 6. — Embryo XXII, six weeks old. Enlarged 5 times.



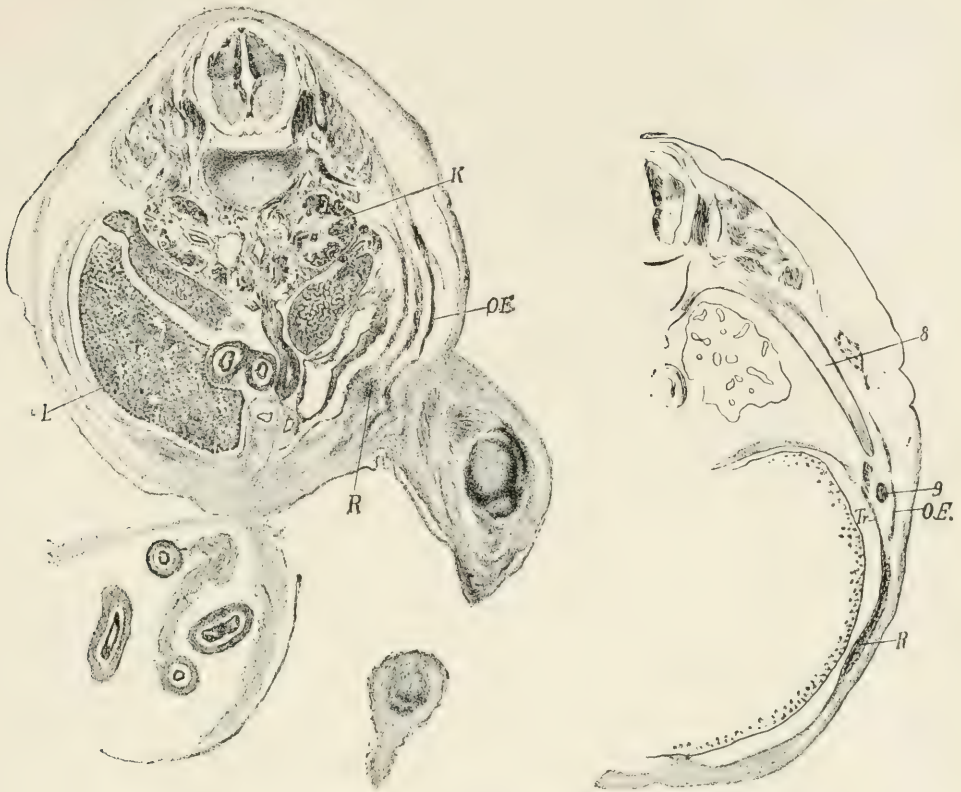
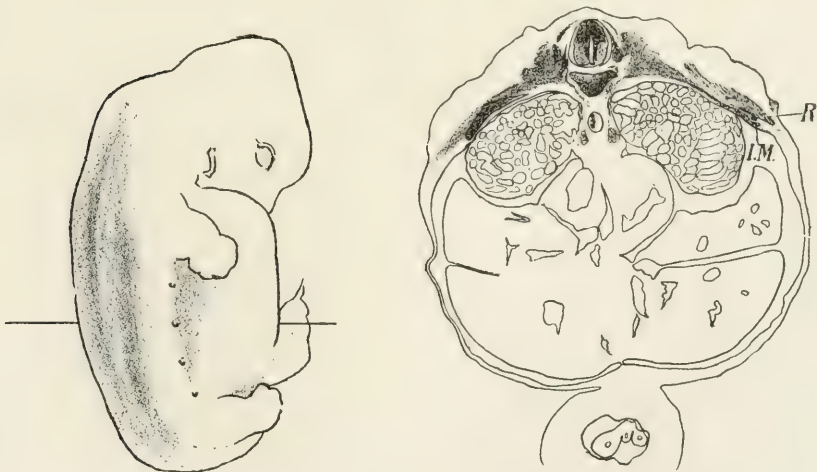


FIG. 7. — Section through lower part of the body of embryo XXII. Enlarged 10 times.

*K.*, kidney; *O.E.*, external oblique; *R.*, origin of the rectus.

FIG. 8. — Section through the middle of the body of embryo XXII. Enlarged 10 times. *8* and *9*, eighth and ninth ribs; *O.E.*, external oblique; *Tr.*, transversalis; *R.*, rectus.

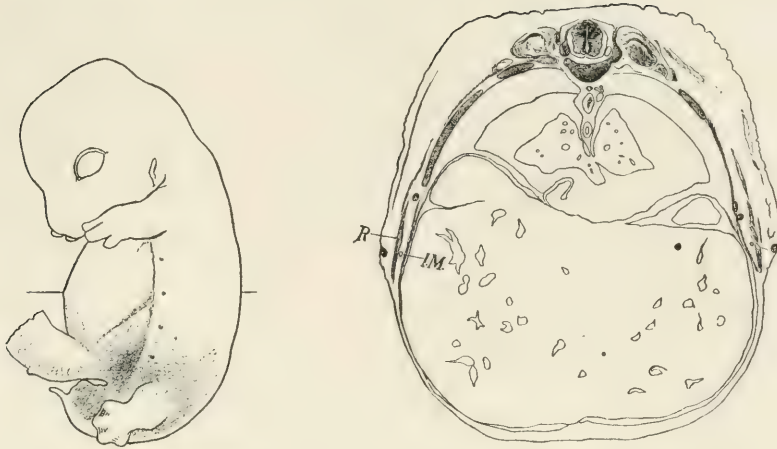


FIGS. 9 AND 10. — Outline and section of a pig's embryo 16 mm. long. *R.*, rectus still attached to the myotome; *I.M.*, internal mammary artery.

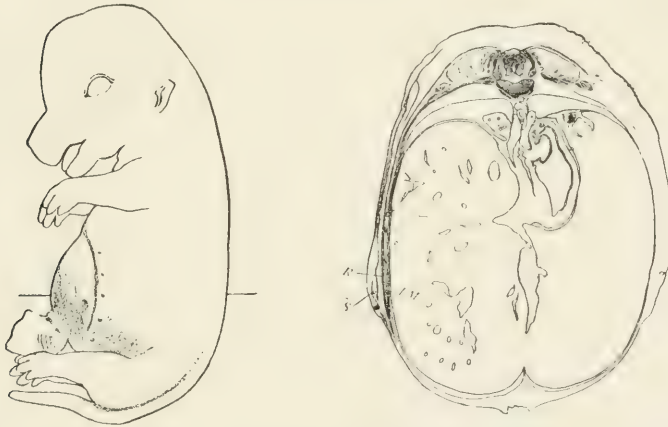
*Schmidt del.*







FIGS. 11 AND 12. — Outline and section of a pig's embryo 23 mm. long. *R.*, rectus; *I.M.*, internal mammary artery.



FIGS. 13 AND 14. — Outline and section of a pig's embryo 33 mm. long. *R.*, rectus; *I.M.*, internal mammary artery. A superficial vessel *S.*, which runs along the milk line, is shown in sections. This vessel is a vein, and can be seen beautifully in the living specimen.

*Schmidt del.*



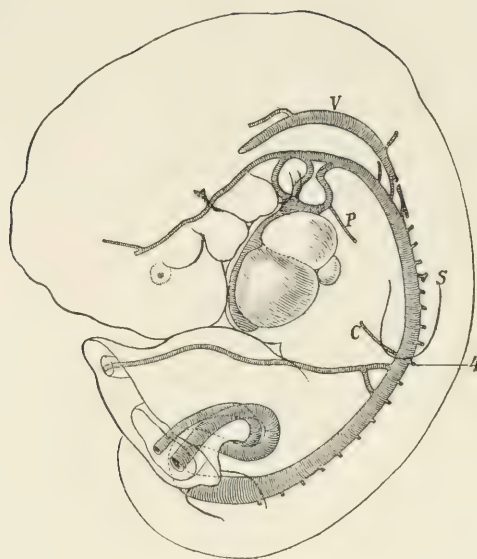


FIG. 15. — Arterial system of embryo II. *A*, fourth dorsal segmental artery; *V*, vertebral  
*P*, pulmonary; *S*, subclavian; *C*, coeliac axis.

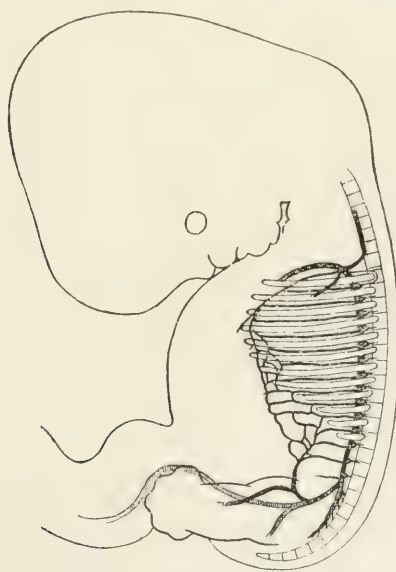


FIG. 16. — Arterial system of embryo XLIII.

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## BUDDING IN PEROPHORA.<sup>1</sup>

GEORGE LEFEVRE.

ALTHOUGH the phenomenon of budding in the group of Tunicata has received the attention of numerous observers for a long time, during the last twelve or fifteen years investigation has been largely confined to the process in the pelagic forms, Salpa, Doliolum, and Pyrosoma. In the ascidians, however, what we have known of the budding, until recently, has been based almost entirely on the observations of earlier workers. Among the latter Kowalewsky (12 and 13), who studied the bud development in Perophora, Didemnum, and Amaroucium, Della Valle (4), in Didemnum, Distaplia, and Botryllus, and finally Seeliger (29) and Van Beneden and Julin (33), in Clavelina, are especially prominent.

Within the last three or four years, however, our knowledge has been greatly enriched by the researches of Pizon (22) and Hjort (8) on Botryllus, of Salensky (27) on Distaplia, and by those of Caullery (1). Although the observations of these investigators agree in many important points, still, in others, notably the origin of the nervous system, great difference of opinion exists.

<sup>1</sup> This paper was accepted as a dissertation for the degree of Doctor of Philosophy by the Board of University Studies of the Johns Hopkins University, May, 1896.



While the Marine Laboratory of the Johns Hopkins University was stationed at Beaufort, N. C., during the summer of 1894, I collected material for the purpose of studying the development of the buds of *Perophora viridis* Verrill, which was found there in great abundance. This material I supplemented the following summer at Woods Holl, Mass., while working in the laboratory of the United States Fish Commission.

My main object in undertaking the investigation was to compare the bud development of *Perophora* with that of *Botryllus*, as described by Hjort (8) and others, and especially to determine, if possible, the origin of the nervous system.

The material, which was easily obtained at both places in large quantities, proved to be most excellent for my purpose, as it contained an unlimited supply of buds in all stages of development.

The preserving fluids used were (1) glacial acetic acid, (2) a mixture of eighty parts concentrated corrosive sublimate solution and twenty parts glacial acetic acid, and (3) Perenyi's fluid. The latter reagent gave perhaps the most satisfactory results, although very good preparations were obtained with the sublimate-acetic mixture, when the objects were left in it not longer than ten minutes.

Mayer's haemalum proved to be most serviceable, while borax carmine gave an excellent stain when used after acetic acid or sublimate-acetic, but was of no value for objects fixed in Perenyi's fluid.

I have made use of Patten's method of orientation (21) with great advantage, and have found it of invaluable assistance in manipulating very small buds, which I have been enabled to cut with accuracy in any plane desired.

In studying the bud development of *Perophora* I have employed serial sections of all stages in the sagittal, frontal, and transverse planes, and also a complete series, throughout the entire development, of buds mounted in various positions as total preparations.

The sections were cut from 3 to 5  $\mu$  in thickness on a Thome microtome, and all the drawings were made with the aid of a Zeiss camera lucida.

I take much pleasure in acknowledging my indebtedness to Professor Brooks for the kindly interest with which he has followed my work, and for valuable assistance given me. I also desire to express in this place my great appreciation of the many courtesies extended to me by the late Colonel Marshall MacDonald at the station of the United States Fish Commission at Woods Holl.<sup>1</sup>

*Perophora viridis* Verrill.

This ascidian, which is the only species of *Perophora* known to occur on the Atlantic coast of North America, was first found in Vineyard Sound by Verrill (34), and described by him in 1871. A new species, *P. annectens*, has recently been reported from the coast of California by Ritter (23).

*Perophora viridis* grows luxuriantly below low-water mark on the wharf piles in Little Harbor, Woods Holl, and Vineyard Haven, Martha's Vineyard. I also found it to be equally abundant during the summer months in the harbor of Beaufort, N. C. The colonies form large, thick clusters of a beautiful greenish-yellow color, and usually occur together with other ascidians and with hydroids, bryozoa, sponges, and barnacles, the delicate stolons creeping over and covering the surfaces of everything within reach.

*The Rudiment of the Bud.*

Budding in *Perophora* was first studied by Kowalewsky (12), whose careful work on this form laid the foundation for all subsequent investigation of the process of budding in the ascidians.

Metschnikoff (18) had already discovered in *Botryllus* that the bud-rudiment consists of two vesicles, one within the other, the outer being derived from the ectoderm and the inner from the peribranchial wall of the parent. He also observed the splitting up of the inner vesicle to form the median branchial sac and the lateral peribranchial spaces, but neither Metschni-

<sup>1</sup> A preliminary account of some of my results was published in the *Johns Hopkins University Circulars*, No. 119, June, 1895.

koff nor Krohn (15), who confirmed these observations on the Botryllus bud, saw at all clearly the details of the development. This was left for Kowalewsky, who described many of the internal processes occurring in the bud development of *Perophora listeri*. He showed that in *Perophora*, also, the young bud is composed of two vesicles, the outer one being derived from the ectoderm and the inner from the partition or cloison of the stolon. According to Kowalewsky's account, the endodermal or inner vesicle becomes divided completely into three portions, the two lateral fusing dorsally and forming the peribranchial cavity, and the median giving rise to the branchial sac. I shall try to show below that in *Perophora viridis*, at all events, the peribranchial cavity is formed by quite a different process.

The origin of the bud as a double-walled vesicle has been verified by all subsequent investigators, and thoroughly established as a type of development common to all ascidians whose budding has been studied.

The outer wall of the vesicle is directly derived from the ectoderm of the parent animal, and becomes the ectoderm of the bud. According to the majority of investigators, this outer layer takes no active part in the further development, but Salensky (27) and Oka (20), as will be pointed out below, maintain that the ectoderm is concerned in the formation of the nervous system.

As to the derivation of the inner wall of the vesicle, the case is not so simple, for in different ascidians this layer may arise from entirely different parts of the parent, coming in some forms from an endodermal, in others from an ectodermal structure. In *Perophora* and *Clavelina* it is derived from the cloison or septum of the stolon, which in the latter, and presumably in the former, is of endodermal origin; in *Didemnum* and *Distaplia* from the wall of the gut, and in the *Polyclinidae* from the endodermal wall of the post-abdomen.

In all the above-mentioned species, then, the inner vesicle of the bud-rudiment is derived from an endodermal structure. In *Botryllus*, however, this inner vesicle is formed directly as an evagination of the outer wall of the peribranchial sac, whose

origin in the ascidian larva has been a question of much dispute. Kowalewsky (11) first showed that in the larva of simple ascidians the peribranchial sacs are formed as two symmetrical ectodermal invaginations, which later fuse together dorsally and surround the branchial sac. Della Valle (4), however, contradicted these results, and maintained an endodermal origin for the peribranchial sacs in both simple and compound ascidians, and Pizon (22) has recently described the sacs as arising from two diverticula from the branchial sac in the larva of *Botryllus*. Kowalewsky's account is borne out by all the later investigation of the subject, with the exception of that of Pizon. Seeliger (29, *Clavelina*), Willey (36, *Ciona*), Salensky (28, *Diplosoma*, *Didemnum*), and Caullery (1, *Distaplia*, *Leptoclinum*, *Glossophorum*, *Circinalium*) agree in their statements that the peribranchial sacs arise in the manner described by Kowalewsky, and are therefore purely ectodermal. It is fairly safe to assume that Pizon is wrong, and that in *Botryllus*, as in all other ascidians studied, the sacs are ectodermal. If this be the case, as the inner vesicle of the bud-rudiment is directly cut off from the outer peribranchial wall in both embryozooid and blastozooid, it follows that all the organs, including the peribranchial sacs, derived from this inner vesicle throughout the entire series of buds proceeding from the larva are ultimately of ectodermal origin.

After this brief review of the state of our knowledge concerning the origin of the bud-rudiment in the ascidians, I shall now describe the process as I have found it to occur in *Perophora viridis*.

The buds arise in a single row along the course of the delicate stolons, which branch profusely and adhere on one side to the surface over which they creep. The branches come off approximately at right angles to the stems from which they proceed; but aside from this characteristic there is no regularity whatever in the method of branching—they arise at unequal intervals, and as each grows out it gives off in its turn still younger shoots, the whole system becoming much tangled and twisted to form a loose felt-work.

Proceeding towards the growing tips of the stolons, both buds and branches are seen to become successively younger.



In Pl. XXX, Fig. 6, is shown a transverse section of a stolon. The outer wall of the hollow cylindrical tube consists of a flattened epithelium, the ectoderm (*ec.*), covered externally by the transparent cellulose test (*t.*). Running from one side to the other, the septum (*pt.*), is seen in cross-section, completely dividing the tube into two compartments or sinuses (*bl.s.*), in which the free cells of the blood lie scattered about. This stolon partition consists of a double lamella made up of flat, attenuated cells, and may be described as a collapsed cylinder, the walls of which are closely pressed together and attached along the upper and lower borders to the inner surface of the ectodermal tube. It divides the stolon longitudinally into halves, and stretches entirely across the lumen, although in preserved material it is usually more or less shrunken away from the outer wall. The two compartments of the stolon are in open communication with the body cavities of the animals, so that a free circulation of blood occurs from one individual to another.

As Kowalewsky (12) has pointed out, the partition ("Scheidewand") does not reach quite to the extreme distal end of the stolon, but there the two spaces or sinuses open into each other. The blood flows in opposite directions in the two sides of the stolon, up one to the tip, around the free end of the partition, and down the other. When the heart beat is reversed, of course the direction of the blood flow in the stolon is changed.

The buds always arise in the plane of the partition, on the side of the stolon opposite to that which is attached to the underlying surface; the line of attachment is, therefore, parallel to and immediately beneath the lower border of the partition. It will be seen further on that the definitive median plane of the bud coincides with the plane of the partition, and hence the latter structure divides the stolon into a right and left half in reference to the parts of the bud, and not into a dorsal and ventral portion, as described by Kowalewsky (12), whose well-known figure of the *Perophora* stolon is consequently misleading. Looking at the lateral surface of the buds, as shown in that figure,<sup>1</sup> the partition would be seen from the flat side and not on edge.

<sup>1</sup> This figure is reproduced in Korschelt and Heider's *Entwicklungsgeschichte*, p. 1366.



The first indication of the appearance of a bud is a slight bulging out of the ectoderm of the stolon at one point, and if a transverse section be taken at this spot, it will be seen that the two layers of the partition have spread widely apart, giving the appearance of a more or less spherical vesicle in section. This is well shown in Pl. XXX, Fig. 7. The walls of the partition are seen to be no longer composed of flat cells, except on the lower side, that is, the side next the surface of attachment, but have become very much thickened by active cell multiplication. The cells, too, of the ectoderm over the thickened portion of the partition have increased in height and are now nearly cuboidal. The partition, where it is swollen out into a vesicle, becomes drawn away at its lower border from the outer wall of the stolon; I do not think that this is due to shrinkage, for it is invariably found, and, moreover, beyond this region on either side, where the partition is still flat, it is seen to stretch clear across the lumen of the stolon.

The ectoderm continues to bulge out more and more, until it forms a hemispherical protuberance on the surface of the stolon. The cells composing the raised portion of the ectoderm do not remain cuboidal, but from now on, as the swelling increases, become gradually flattened again. The thickened portion of the partition keeps pace with the ectodermal evagination, and grows out into the latter; but the thin walls below now come together, and close off the upper portion as a thick-walled vesicle, without, however, severing the connection. This process is readily understood from Pl. XXX, Fig. 8, in which the walls of the lower part of the partition (*pt.*), have become united again.

In Pl. XXIX, Fig. 1, a bud at about this stage is shown from the exterior.

As the ectoderm continues to push out, it becomes constricted where it passes over into the stolon ectoderm, the constriction being greater at first before and behind than on the sides. The bud-rudiment, which by this time is almost spherical, is sharply marked off from the stolon, and stands either straight out as a round knob from the surface of the latter or is slightly inclined towards its free tip (Fig. 2).

It now represents the characteristic double vesicle of the young ascidian bud; the outer or ectodermal vesicle is directly derived from the ectoderm of the stolon, and the inner or endodermal vesicle, which has become a closed sac, arises by evagination of the thickened dilated portion of the stolon partition. The connection between the partition and the inner vesicle is retained for a long time, and the cavity of the latter is to be regarded as being in communication with the potential cavity of the partition.

A transverse section at this stage (Pl. XXX, Fig. 8) shows the still somewhat cuboidal cells of the ectoderm of the bud-rudiment, and the endodermal vesicle (*in.v.*), with its much thickened walls lying inside. Active cell multiplication has been going on in the latter, which are of nearly uniform thickness, except below, where they pass off gradually into the thin walls of the partition. The cells of the blood are found scattered about between the two vesicles, and it is to be especially noted that many are lying close against the inner surface of the ectoderm and outer surface of the endoderm at numerous points (*m.c.*).

The bud-rudiment does not long remain spherical, but soon becomes elongated by a growth towards the apex of the stolon. It now assumes an oval shape, and one end lies free over the surface of the stolon, beyond the portion which is directly connected with the latter (Pl. XXIX, Fig. 3). It is the free end which is later to be distinguished as the anterior portion of the bud, while by this process of elongation anteriorly the connection with the stolon comes to be left behind in the posterior region. The side next the stolon wall becomes the ventral surface of the bud, and that opposite it the dorsal.

The protuberances of the stolon ectoderm, which Seeliger (29) describes as occurring at the base of the bud-rudiment in *Clavelina*, are not present in *Perophora*; they are merely simple ectodermal sacs filled with blood cells, and do not contain an evagination of the partition. Seeliger calls them "Nahrkammern," and regards them as reservoirs of food material for the developing buds.

Before going on to describe the origin of the various internal

organs, some of which have by this time begun to make their appearance, I wish to say a word about the partition of the stolon.

As the inner vesicle of the bud is formed entirely from this structure, it is a matter of importance to know from what it is derived in the larva. It has never been observed in *Perophora* how the stolon partition arises, although it is usually supposed to be of endodermal origin from its likeness to the similar structure in *Clavelina*. Van Beneden and Julin (33) have shown that in the larva of *Clavelina* it is formed as a direct continuation of the epicardium, which arises as a diverticulum from the posterior wall of the branchial sac, and is, therefore, entirely endodermal. During the month of August, 1895, I made an attempt to discover the origin of the stolon in *Perophora*. Larvae were put into aquaria, through which water was kept constantly flowing, and, although they settled down and underwent the metamorphosis, at the expiration of nearly four weeks not the trace of a stolon sprouted from them. When larvae and young embryozooids were afterwards sectioned and studied, nothing like an epicardium, such as occurs in *Clavelina*, was found. I am unable, therefore, to throw any light on the origin of the stolon partition in *Perophora*, but it is fairly safe to say that it does not arise in the same way as it does in *Clavelina*.

#### *The Further Development of the Bud.*

At the time when the bud-rudiment begins to elongate, or very shortly after, the rudiments of several new structures are laid down. These are (1) the *pericardium*, (2) the *peribranchial sacs*, (3) the *dorsal tube*, (4) the *gut*, and (5) the *ganglion*. They do not all arise simultaneously, and, although the rudiment of the pericardium is the earliest to appear, it will be necessary to describe the formation of the peribranchial sacs first in order to render intelligible certain relations between these and other structures.

*The Peribranchial Cavity.*

All investigators agree in deriving the peribranchial cavity from the inner vesicle of the bud-rudiment, but the manner in which it arises is not the same in all species of ascidians.

In *Perophora*, *Didemnum*, and *Amaroucium*, according to Kowalewsky (12 and 13), two parallel longitudinal furrows appear on the outside of the inner vesicle, and by gradually deepening finally divide the latter completely into three portions. The two lateral divisions which are thus cut off grow up over the middle one, and fuse to form the median portion of the peribranchial cavity, which now surrounds the branchial sac dorsally and laterally.

Seeliger (29) has described a different method of formation of the peribranchial cavity in *Clavelina*. According to him, the inner vesicle is not divided into three portions, but into two, one of which, the posterior, gives rise to the branchial sac and the gut, while the other forms the whole peribranchial cavity. These results were contradicted by Van Beneden and Julin (33), who maintained that in *Clavelina* the process is the same as that described by Kowalewsky. As Seeliger's view was not founded on an investigation of an uninterrupted series of stages, and as there were wide gaps in his observations at periods which are especially concerned in the formation of the peribranchial cavity, the supposition that his results are wrong is very probable.

In *Distaplia* Salensky (27) has shown, and his results have been confirmed by Hjort and Frl. Bonnevie (10), that the inner vesicle gives rise to two lateral evaginations, which become completely constricted off as separate vesicles, the peribranchial sacs, and, gradually extending dorsally, fuse together on the median line. These sacs are not formed at the same time, but one is given off from the inner vesicle before the other, so that at a very early stage an asymmetry of the bud is produced.

Della Valle (4) described a similar method of formation of the peribranchial cavity for *Botryllus*, but both Pizon (22) and Hjort (8) have conclusively proved his observations to be erroneous. According to these latter authors, both the lateral



and median portions of the peribranchial cavity in this ascidian arise at the same time as a saddle-shaped bag, which is cut off by two longitudinal furrows from a median vesicle, the later branchial sac. Hjort regards this process as a great curtailing of the embryonic development, such as often takes place in buds. Salensky (27) confirms this conclusion, but goes a step further, saying that "die Entwicklung der Peribranchialhöhlen des Botryllus eine Abkürzung nicht nur bezüglich der embryonalen Entwicklung, sondern auch bezüglich der Entwicklung dieser Organe in den Knospen anderer Ascidien darstellt," *Distaplia*, for example. My observations on the development of this structure in *Perophora viridis*, although agreeing with those of Hjort in so far as they show that the peribranchial sacs do not arise separately as closed vesicles which later unite to form the cloacal cavity, indicate that the process is not so simple as that which occurs in *Botryllus*.

If a transverse section of a bud be examined about the time when the elongation spoken of above is just beginning, it will be found that the wall of the inner or endodermal vesicle is no longer of uniform thickness. Pl. XXX, Fig. 9, is drawn from such a section. The ectoderm covering the bud, although it is not shown in the figure, has again become flattened after its temporary thickening, and is now like that of the stolon. The figure clearly shows that the stolon partition is made up of two lamellae, which are continuous below with each other and pass over above into the walls of the inner vesicle.

The important change to be noted, however, is that the wall of the endodermal vesicle on one side, the left, is getting perceptibly thinner than elsewhere, and that the whole vesicle is no longer symmetrically placed with reference to the stolon partition, but is bulging out slightly to the right. This is the first indication of a marked change which is about to take place in the internal relations of the bud-rudiment.

By a peculiar process, which may be described as a transverse or rotatory growth affecting the inner vesicle, the thicker wall of the right side (Fig. 9, *r.w.in.v.*) is carried or pushed down gradually until it comes to lie eventually on the ventral side, that is, the side next to the stolon.



The stolon partition remains stationary, and the displacement or shifting around of the inner vesicle takes place on this as a fixed support. The process might be illustrated by the drooping of a flower to one side on its stem, although the change of position cannot be a purely passive falling over of the vesicle. In Fig. 9 a small collection of cells (*pc.r.*) is seen adhering to the wall of the vesicle high up on the right side, and these, as we shall see below, form the rudiment of the pericardium. This cell mass remains fixed at the same place on the wall, and during the shifting of the vesicle is borne down towards the ventral side, describing in its descent an arc of about  $90^{\circ}$ . It therefore furnishes a good register of the progress of the displacement of the vesicle.

As the turning proceeds, the difference in thickness between what was at first nearly the whole right side and the rest of the vesicle becomes more marked; consequently, the cells composing the entire vesicle, except in the thicker region, are seen to be growing more and more flattened.

The displacement is most probably brought about by a rapid growth and flattening of the cells composing the greater portion of the vesicle, whereby the actual right side, which is morphologically the ventral side of the vesicle, is shifted or pushed ventrally through  $90^{\circ}$ .

This process is analogous, at all events, with the rotation or displacement of the pharynx of the *Amphioxus* larva from right to left, although I am not prepared to claim any phylogenetic relation between the two.

By comparing Fig. 9 with Figs. 10 and 11, Pl. XXX, the process can be readily understood. The shifting, however, involves the anterior end of the vesicle only to a slight extent. In this region a difference in thickness of the walls is not observed, and the rudiment of the dorsal tube, which definitively has a median dorsal portion in the anterior end of the bud, arises as a collection of cells almost at the same time as the pericardial rudiment appears, lying a little to the *left* of the mid-dorsal line on the wall of the vesicle (Fig. 13, *d.t.r.*). If the displacement took place to as great an extent anteriorly as posteriorly, it is evident that this cell mass would appear much

further down on the left side; but that the anterior end is slightly rotated is shown by the fact that the rudiment appears not exactly in the median line, where it is eventually brought through the shifting of the vesicle, but somewhat to the *left* of it.

The formation of the peribranchial cavity is associated with this change of position of the endodermal vesicle. In Figs. 10 and 11 it is seen that the lower portion of the vesicle at the point indicated by the line *a* is being bent in, with the result that the wall in this region makes two angles, one directed inward and the other outward (Fig. 11, *a* and *b*). The apex of the latter marks a point on the wall of the vesicle which will have traveled through 90° when the displacement is completed, as its final position will be in the mid-ventral line.

As the inwardly directed fold (Fig. 11, *f.l.pb.s.*) deepens, it gradually divides off a portion of the inner vesicle on the left side, which is connected with the stolon partition; this is the left peribranchial sac (Pl. XXXI, Fig. 20, *l.pb.s.*). This fold begins somewhat in front of the middle of the vesicle, and, deepening rapidly in this region, gradually extends posteriorly.

As these changes are going on, the connection with the stolon partition is gradually becoming constricted, and is now only present in the posterior half of the bud, while at the same time the ectodermal stalk is also getting narrower. Ritter (24) in a preliminary note on the budding of Perophora says that "when the differentiation of the 'endoderm' into the branchial and two peribranchial sacs takes place, it does so in such a way that the developing blastozooid is connected with the double-walled partition of the stolon, not by the branchial sac, as has been hitherto supposed, but by the left peribranchial sac." He does not, however, describe how this comes about. From an examination of Pl. XXX, Figs. 9, 10, and 11, it is readily understood. The communication of the body cavity of the bud with the blood spaces of the stolon is never completely closed, as there is always a free circulation of blood from the one to the other; but eventually the left peribranchial sac is entirely severed from the stolon partition. I cannot, however, confirm Ritter's statement (*loc. cit.*, p. 367) that this connection is

lost at an early stage, namely, "at a time when the two peribranchial pouches have merely begun to envelop the branchial sac." I find that it persists for a very much longer time, and is still present, although greatly constricted, at a stage when some of the gill slits have been formed and the peribranchial cavity has been wholly separated from the branchial sac. Pl. XXXI, Fig. 24, shows the connection (*r.st.c.*) at such a stage.

The first indication of the right peribranchial sac is a slight longitudinal folding-in of the wall of the inner vesicle some distance up on the right side, which appears after the shifting of the vesicle has begun. This furrow starts a little in front of the anterior termination of the left peribranchial fold, and as it deepens and extends posteriorly, it is gradually carried down towards the ventral side, in the same way as the pericardial rudiment. It is already present at the stage represented in Pl. XXX, Fig. 11, but has not yet reached back far enough to appear in a section which shows the left fold. In Fig. 12, which is taken from the same series of sections, but a little further forward, it is well marked (*f.r.pb.s.*).

As the shifting continues, the inner vesicle tends more and more to assume a symmetrical position. The two peribranchial furrows, which deepen rapidly and run in obliquely to meet each other, do not come together on the dorsal surface of the vesicle, but some distance below it. The result of this is that when the right and left peribranchial sacs are separated from the inner vesicle a median dorsal portion connecting them is cut off at the same time. This median piece, hence, does not arise, as Kowalewsky (12 and 13) describes, from the fusion of the lateral sacs dorsally, but the three portions are formed by one and the same process. We now find a median vesicle, the later pharynx, surrounded dorsally and laterally by a saddle-shaped bag which consists of the dorsal or cloacal and the lateral divisions of the peribranchial cavity. This is essentially the same process as that which Pizon (22) and Hjort (8) have described for *Botryllus*.

In *Perophora* the folds which separate the peribranchial cavity from the inner vesicle do not involve the entire length of the latter, but leave nearly the whole of the anterior half undivided,

as well as a short region at the posterior end of the vesicle. And, further, the whole peribranchial cavity is not constricted off at the same time, but, as stated above, the furrows begin anteriorly and extend gradually back, so that at any given stage the opening of the median vesicle into the peribranchial cavity is much wider in a posterior section than in one further forward.

When the right and left peribranchial sacs are being formed, as just described, a broad pouch or diverticulum grows out from the anterior margin of each, and by degrees spreads over the undivided portion of the original inner vesicle. These pouches are direct continuations of the lateral cavities, and later completely cover the sides of the anterior region of the peribranchial sac, but *they never fuse dorsally*.

Similar extensions are carried out from the posterior margin of the lateral cavities, and though not prominent at first, still, as the bud gets older and increases in length, they attain a considerable size and surround a part of the digestive tract.

The peribranchial cavity now consists of two deep lateral sacs, surrounding the spacious branchial sac, and connected dorsally by a median space, the cloacal cavity or atrium. The lateral sacs are unsymmetrical, however, until quite a late stage, for the *anterior* pouch of the *right* peribranchial sac grows more rapidly and extends further forward than the similar pouch on the left side, while the *posterior* pouch of the *left* side extends further back than the corresponding one on the right. Eventually the two sacs become symmetrical.

The formation of the peribranchial cavity is easily understood from the series of sections represented in Pl. XXXI, Figs. 16-21; these will be rendered more intelligible by a comparison with Pl. XXIX, Fig. 4, which is drawn from a total preparation of a bud at the same stage of development. The sections are taken respectively at the levels indicated by the parallel lines, *a, b, c, d, e, and f*, of Fig. 4. In Pl. XXXI, Fig. 16, line *a* of Fig. 4, the most anterior one of the series, the branchial sac (*br.s.*) is seen by itself, for the extensions of the peribranchial sacs have not reached far enough forward to appear in the section; the hypophyseal tube (*d.t.*) is shown on the dorsal side



of the branchial sac. Fig. 17, line *b*, only includes the anterior extension of the right sac (*r.a.ex.*), for, as just stated above, the pouch on the opposite side lags behind in its growth. Fig. 18, line *c*, represents a section taken just in front of the anterior face of the cloaca, and shows both peribranchial sacs at the level where they are continued forward into their anterior extensions. In Fig. 19, line *d*, the section passes through the anterior portion of the cloaca (*cl.*), which is seen to connect the lateral sacs; the constriction, which will ultimately completely separate the saddle-shaped bag from the median vesicle, has proceeded in this region to a considerable extent, and has greatly narrowed the opening between the peribranchial and branchial cavities. In a section further back (Fig. 20, line *e*), the folds which are forming the peribranchial sacs are much less deep and wider apart; the connection between the left sac and the stolon partition is present in this region (*st.c.*). Finally, Fig. 21, line *f*, represents a section beyond the peribranchial sacs, the posterior pouches of which at this stage have not yet begun to grow out; the section passes behind the connection with the stolon partition, but through the intestine (*int.*), which is seen on the left side.

The further development of the peribranchial cavity from this stage on merely consists in the completion of the constriction, whereby the saddle-shaped bag is completely cut off from the branchial sac, and in the extension of the anterior and posterior pouches of the peribranchial sacs, which finally surround the whole pharynx laterally. These relations are illustrated by the series of sections (Figs. 22-24), which are taken from the same bud. Fig. 22 is a section through the anterior end of the bud, and shows the lateral extensions of the peribranchial sacs (*r.a.ex.* and *l.a.ex.*) surrounding the pharynx (*br.s.*). The peribranchial cavity is now entirely cut off, and its lateral portions are united in the middle region of the bud by the dorsal connecting piece or cloaca; this condition appears in Fig. 23. Beyond the cloaca the posterior pouches of the lateral sacs, which, like the anterior pouches, are not united dorsally, are seen in Fig. 24 (*r.p.ex.* and *l.p.ex.*); the connection of the left sac with the stolon partition in this figure has already



been referred to. By this time the process of displacement is completed, and the definitive symmetrical arrangement of the pharynx and peribranchial cavity is reached. The connection between the left peribranchial sac and the partition of the stolon is nearly severed; it is found in only two sections of this series, one of which is seen in Fig. 24. A total preparation of a bud at about this stage is shown in Pl. XXIX, Fig. 5, which may be readily compared with these sections.

### *Epicardium.*

This structure was first described by Van Beneden and Julin (33) in the buds and larvae of *Clavelina*, and was shown by these authors to be closely connected with the development of the pericardium. It arises as an evagination of the posterior wall of the branchial sac, and a little further back divides into two blind pouches, which remain separate in the buds, but in the embryo unite to form the "cul de sac epicardique" of Van Beneden and Julin; the latter is continued into the stolon to form the double-walled partition. The development of the epicardium will be again referred to in connection with the pericardium, with which it stands in very close relation in some ascidians.

In *Distaplia*, Salensky (27) has described the epicardial sacs as arising in the buds at an early stage by evagination from the posterior end of the inner vesicle; the two sacs are not formed at the same time, and the left one is always larger than the right.

In the buds of the *Polyclinidae* the epicardium is formed in the same way; two small diverticula, a right and a left one, are given off from the posterior end of the branchial sac, from which they afterwards become detached. They soon, however, unite to form a single tube, which is continued out into the post-abdomen, where it is destined to furnish the inner vesicles of the buds produced by transverse constriction of that region of the body.

The existence of an epicardium in *Botryllus* is denied by Hjort (8), but maintained by Pizon (22). According to the

latter, the inner vesicle at a very early stage gives off two anterior lateral diverticula, one on each side, which later form the peribranchial cavity and also two posterior lateral diverticula. These four pouches are at first separate, but soon the two on each side fuse in the middle region of the bud. When the peribranchial cavity is separated from the inner vesicle, the posterior diverticula are cut off at the same time, and now appear as posterior prolongations of this cavity, with which they always remain in free communication. They are what Pizon calls the "*diverticules périviscéraux*," and in later stages completely envelop the digestive tract. From the fact that these pouches arise as two diverticula from the posterior end of the inner vesicle, Pizon regards them as homologous with the epicardial tubes of other ascidians, and states (*loc. cit.*, p. 29) that "la formation de cette cavité périviscérale n'est pas secondaire et qu'elle s'est annoncée, dès le début, par deux petits diverticules postérieurs de la vésicule primitive, en même temps que les diverticules antérieurs correspondants qui engendreront la cavité péribranchiale."

These perivisceral diverticula, however, differ from the epicardial tubes of *Clavelina*, *Distaplia*, and the *Polyclinidae* in that they communicate with the peribranchial cavity.

Hjort (*loc. cit.*, p. 594) states that the "einheitliche Peribranchialblase sich nun derart weiter entwickelt, dass sie nicht nur den Abschnitt des Kiemendarmes, sondern den ganzen Darmtractus unwächst," and Salensky (27), who accepts the conclusion of Pizon as to the homology of the perivisceral diverticula, thinks that Hjort evidently saw the "epicardial sacs" in *Botryllus* but failed to recognize them as such. Salensky believes that the connection of the "epicardial sacs" with the cloaca in *Botryllus* must be regarded as a result of the early separation of the peribranchial cavity from the inner vesicle.

In the light of these considerations it is possible that the posterior extensions of the peribranchial sacs, which I have described as arising in the buds of *Perophora viridis*, are likewise homologous with the epicardial sacs of other ascidians. It is to be remembered, however, that if such be the case, which

I think doubtful, their direct origin from the inner vesicle has been completely lost, as they do not appear until quite a late stage, and then merely as prolongations backward of the lateral portions of the peribranchial cavity, after the latter have been entirely cut off from the inner vesicle. This would, therefore, be a still more modified condition than that which is found in *Botryllus*.

Pizon (*loc. cit.*, p. 105) makes the statement, which is not, however, illustrated by figures, that he has confirmed on the buds of *Perophora listeri* the results of Kowalewsky (13, *Amaroucium proliferum*) and of Van Beneden and Julin (33, *Clavelina rissoana*) in regard to the origin of the epicardial tube. "Ce tube," he says, "résulte bien de la réunion de deux petits diverticules qui naissent à droite et à gauche du sac branchial et qui s'isolent complètement de celui-ci à un moment donné." Such a description is not in the slightest accord with my observations, and if an epicardial tube arises in this manner in the buds of the European *Perophora*, it certainly does not in *Perophora viridis*.

#### *The Branchial Sac or Pharynx.*

That portion of the original inner vesicle which is left after the separation of the peribranchial cavity becomes the pharynx. At its anterior end it finally opens to the exterior through the branchial orifice, and after the appearance of the gill slits communicates with the peribranchial cavity, while posteriorly it leads off into the digestive tract.

The formation of the branchial sac in the buds of *Perophora viridis* is complicated by reason of the peculiar shifting of the inner vesicle, which has been described above. The whole vesicle, with the exception of the anterior end, which, as already stated, is but slightly involved in the process, becomes shifted or revolved through about 90°, in such a way that the original right wall of the vesicle comes to lie ultimately on the ventral side. This right wall, as has been shown, is early found to be much thicker than the rest of the vesicle, the difference being due, not to an increase in thickness of this region, but to the

flattening of the cells composing the remaining portion of the vesicle. It is this thickened wall, originally on the right side, which forms the floor of the pharynx in that part of the vesicle which is concerned in the displacement. Very soon after the beginning of the change in position, a shallow longitudinal groove is found on the inner surface of the vesicle in the middle region of the bud, lying on the right side on a level with the lower border of the pericardial rudiment. This is the first appearance of the *endostyle*; its position is shown at *end* in Pl. XXX, Fig. 11, but at an earlier stage it is found much higher up. In this figure and the next one it is seen that the groove runs through about the middle of the thickened area, that is to say, above and below it there are equal portions of the thick wall, which will lie to its right and left when the change in position of the vesicle is fully accomplished.

The groove rapidly extends anteriorly and posteriorly, and at the same time becomes deeper and broader. When it reaches its definitive position in the mid-ventral line, it stretches throughout the entire length of the branchial sac.

It will not be necessary to speak of the differentiation of the endostyle into the various zones of cells which go to make it up, as these have been described by numerous authors,—Della Valle (3), Herdman (7), Lahille (17), and others.

It is to be especially noticed in Figs. 10, 11, 20, and 24 that the positions of the pericardial rudiment and endostylar groove in reference to each other remain the same during the displacement of the vesicle. From this fact it is evident that the thick portion of the vesicle is carried down bodily, and that no interstitial growth takes place in this region during the process, else the distance between the pericardium and endostyle would not remain the same. It cannot be said that the pericardial rudiment might compensate by its own growth for any increase in extent of that part of the wall against which it lies, for it covers practically about the same area as long as it adheres to the vesicle. It would seem, therefore, that the change in position of the inner vesicle is brought about by the stretching out and flattening of the cells in all but the thick area, and that the latter is borne or rather pushed down toward the ventral side.



*The Branchial Stigmata.*

The branchial stigmata or gill slits are not formed until after the peribranchial cavity has been completely separated from the branchial sac. The first to appear lie far back towards the posterior end, but very soon they begin to break out in spots all over the sides of the branchial sac.

The tendency to arise in vertical rows becomes apparent when only very few are present, but each slit is a separate and independent formation. I have never observed the origin of one slit from another, such as occurs in the larvae of ascidians.

Fig. 5, Pl. XXIX, represents a stage when about eight slits have been formed on each side; as the anterior pouches of the peribranchial sacs grow further and further forward, new rows of slits are laid down along their free margins.

The first indication of a gill slit is a small, circular, thickened area of the branchial wall, which at this spot becomes slightly evaginated until it touches the visceral wall of the peribranchial sac (Pl. XXXII, Fig. 29 *a*, *g.s.r.*). The cells of the latter at this point become thickened somewhat, and now a fusion takes place between the two walls; this is seen in Fig. 29 *b* (*g.s.r.*). The opening, which breaks through the center of the fused patch of cells, is drawn out later in the long axis of the bud into a narrow slit, which is provided with cilia in the usual way. The upper part of Fig. 29 *b* shows a slit just after the opening has been formed (*g.s.*).

*The Branchial and Cloacal Orifices.*

The branchial orifice arises at a tolerably late stage, and is first indicated by a great increase in thickness of the ectoderm at a point opposite the extreme anterior end of the branchial sac. This thickened area becomes invaginated until the bottom of the pit touches the endodermal wall, and a complete fusion of the two soon takes place (Pl. XXXII, Fig. 28 *a* and *b*, and Pl. XXIX, Fig. 5, *e.br.o.*). The cells in the center of the fused area break down, and the cavity of the pharynx is put into communication with the outside. As is shown in Fig. 28



*a* and *b*, many mesodermal cells (*ml.c.*) attach themselves to the inner surface of the ectodermal depression, become greatly elongated, and are eventually transformed into muscle fibers.

In *Botryllus*, according to Pizon (22), it is the branchial wall which thickens and evaginates to fuse with the ectoderm, while the latter plays but a small part in the production of the orifice. The process, as it occurs in *Perophora viridis*, is quite similar to that described by Kowalewsky (11) for *Phallusia*, and by Van Beneden and Julin (33) for *Clavelina*.

The cloacal orifice is formed in exactly the same manner, by the union of an ectodermal invagination with the dorsal wall of the cloaca (Pl. XXIX, Fig. 5, and Pl. XXXI, Fig. 23, *e.cl.o.*).

It will not be necessary to speak here of the various appendages and ciliated growths of the pharynx which arise later, — namely, the tentacles, papillae, languets, dorsal lamina, and peripharyngeal bands, — as these are merely differentiations of the pharyngeal epithelium, and have been sufficiently described by numerous authors.

### *The Digestive Tract.*

Some time before the displacement of the inner vesicle is completed, and when the folds which will cut off the peribranchial cavity are not very deep, the wall of the inner vesicle high up on the left side at the extreme posterior end becomes much thickened, and soon evaginates to produce a little blind pouch, the rudiment of the digestive tract (Pl. XXX, Fig. 15, *gt.r.*). This lateral diverticulum grows out as a tube, which at once bends sharply downwards and forwards, while, as the shifting of the vesicle continues, its opening into the latter is carried up nearer and nearer the mid-dorsal line, where it will ultimately come to lie. The tube soon turns abruptly on itself to form a close U, and, now growing upward along the outer wall of the left peribranchial sac until it reaches the dorsal surface, finally bends directly forward, and stops short at the posterior wall of the cloacal cavity. At this point the distal extremity of the tube fuses with the cloacal wall, an opening breaks through, and the anus is established. The differentiation into

oesophagus, stomach, and intestine takes place very early, and is apparent at a stage considerably younger than that shown in Pl. XXIX, Fig. 4. The course and development of the tube are sufficiently illustrated by Figs. 4 and 5.

As the bud grows and increases in length, the digestive tract enlarges enormously, the U becomes opened more and more, and the intestine describes a wide curve which lies well forward against the outer wall of the left peribranchial sac (Fig. 5). With the anterior extension of the digestive tract and the posterior prolongation of the left peribranchial sac, the whole tract, which lies entirely on the left side of the bud, comes eventually to be closely enveloped by the outer wall of the peribranchial cavity.

The "pyloric gland" or "organe réfringent" of Giard arises as a tubular diverticulum from the lower anterior face of the enlarged portion of the digestive tract which will become the stomach. Before reaching the intestine the tube bifurcates, and each branch in its turn gives off two others, which also divide, the whole system of dichotomously branching tubules finally forming a lace work surrounding the whole intestine.

The development of this problematical organ is already well advanced at the stage shown in Pl. XXIX, Fig. 5 (*o.r.r.*). This figure, together with Pl. XXXII, Fig. 30, which shows a portion of the stomach wall (*st.w.*), leaves no doubt that the tube is directly derived from the digestive tract. This origin was maintained by Della Valle (3), but denied by Roule (25), who stated that the "organe réfringent" is not a part of the digestive tract, but communicates with the heart, and therefore belongs to the vascular system, an opinion already held by Kupffer (16).

Della Valle's view is also supported by Pizon (22), whose description of the development of the organ in *Botryllus* agrees minutely with my observations on *Perophora*.

The terminal branches of the system of tubules which ramify over the surface of the intestine end in little enlargements or ampullae, the walls of which are very thin, and lie closely pressed against the intestinal wall. The cells of the duct are cylindrical, and gradually pass over into the flat cells

of the ampullae. I have failed to find any cilia on the latter, as Chandelon (2) has described in *Perophora*. Pl. XXXII, Fig. 31, shows a cross-section of the intestine (*int.*), surrounded by the thin-walled tubules and ampullae (*amp.*), the flat cells of which contain very deeply stained nuclei. On one side of the figure one of the ducts is cut longitudinally, just where it forks near the surface of the intestine (*o.r.d.*).

Different views have been held concerning the function of the "organe réfringent." Krohn (15), Kupffer (16), and Giard (6) have regarded it as a renal organ; but as the ampullae always contain a clear, unstainable fluid, and never concretions or epithelial débris, this view has been discarded. A second hypothesis, that it is a digestive gland which gives its secretion to the intestine, has been held by Chandelon (2) and Della Valle (3), the latter attributing to the organ an hepato-pancreatic function. Pizon (22), however, believes that the flat cells of the ampullae possess no glandular characters, and cannot be reconciled with a secretory function; but he is inclined to regard the organ as a *chyliferous apparatus*. He says (*loc. cit.*, p. 96): "Je suis plutôt porté à croire que l'épithélium des ampoules ne sécrète rien, et qu'il se charge simplement d'absorber les produits de la digestion qui sont assimilables et qui n'ont pas été pris par les parois de l'intestin. Ces produits quitteraient ensuite la cellule pour aller se mélanger au sang, dont les corpuscules sont précisément extrêmement nombreux autour des ampoules terminales." Although Pizon's hypothesis would seem the most probable one, as the histological structure of the organ is not such as to suggest a glandular function, still, the rôle played by the "organe réfringent" must remain uncertain until the nature of the liquid contained in the tubules is determined.

#### *The Pericardium and Heart.*

Concerning the origin of the common rudiment of the pericardium and heart, investigators have given widely divergent accounts, some deriving it from endoderm, others from mesoderm. Although it is very certain that this structure arises

differently in different ascidians, still, in the buds of one and the same form statements of authors are at variance.

Seeliger (29) describes the pericardium as arising in the buds of *Clavelina* from an enormously large evagination of the ventral portion of the branchial sac, which later becomes separated as an independent vesicle. He did not, however, distinguish the epicardial sacs, and mistook a part of the latter for the pericardium. Van Beneden and Julin (33) showed conclusively that the diverticulum of the branchial sac, observed by Seeliger and called by him the pericardium, is merely a part of the stolon partition wall and is not concerned in the formation of the heart. According to the Belgian authors, who described in detail the development of the pericardium in the buds of *Clavelina*, the pericardium and epicardium at first form a common cavity with the inner vesicle. A separation takes place later in such a way that the epicardium remains in communication with the inner vesicle, while the pericardium becomes entirely cut off from the latter, but retains its connection with the stolon partition. Van Beneden and Julin maintain that the union of the epicardium with the branchial sac is never lost in the bud development of *Clavelina*, and, therefore, do not agree with Seeliger's statement that the diverticulum, which he observed and erroneously regarded as the pericardium, becomes separated from the branchial sac. Seeliger's description of the early constriction of the inner vesicle from the stolon partition is not confirmed by the Belgians, who showed that the pericardium, originally a part of the inner vesicle, preserves its connection with the partition wall, as explained above.

A somewhat similar origin of the pericardium, together with the epicardium from the inner vesicle, is stated by Pizon (22) to occur in the *Polyclinidae*; for example, in *Circinalium* and *Amaroucium*.

Our knowledge of the derivation of this structure in the buds of *Botryllus* is very much less certain. Pizon (22) declares that the pericardium arises as a little diverticulum from the lower wall of the inner vesicle, which becomes completely constricted off as an elongated tube. His conclusion as to the endodermal



origin of the peribranchial rudiment cannot, however, be unhesitatingly accepted, since his figures do not satisfactorily establish the correctness of his description, while the supposition that he has not followed the development with sufficient care is very strong. Salensky (27, p. 527) calls attention to the fact that the little circle of epithelial cells which Pizon marks with the letters *Per* in Pl. I, Fig. 7, "wohl auch einen Querschnitt der unteren Wand des Kleimendarmes darstellen kann," and that it is not at all proved that it is the same structure as the pericardium, figured in later stages.

The first appearance of the pericardial rudiment observed by Hjort (8) in *Botryllus* was a small clump of cells lying against the ventral wall of the inner vesicle in the posterior part of the bud to the right of the middle line. As to the derivation of these cells, Hjort was unable to say whether they were mesodermal cells or cells which had wandered out from the endoderm, but he distinctly states that an evagination of the inner vesicle does not occur at this point.

In the buds of *Distaplia*, Salensky (27) observed a similar collection of cells lying against the lower wall of the branchial sac and surrounded by mesodermal cells. He maintains that there is no ground for attributing an endodermal origin to the rudiment, which is from the beginning sharply marked off from the wall of the branchial sac, and he therefore concludes that the pericardium is derived from the mesoderm.

The result to which my observations on the bud development of *Perophora viridis* have led me, in regard to the origin of the pericardial rudiment, is in accord with that of Salensky.

At about the stage represented in Pl. XXX, Fig. 9, a very loose patch of cells (*pc.r.*) is found applied to the outer surface of the inner vesicle high up on the right side in the posterior end of the bud. Before this time many isolated cells are seen adhering to the wall of the vesicle at numerous points (Figs. 7, 8), but when the difference in thickness between the right side and the rest of the vesicle is just becoming apparent a marked tendency in the scattered cells to accumulate in one spot is noticed. At first there is but a single layer of cells joined loosely together end to end and forming a somewhat elongated



patch; this is the rudiment of the pericardium, which is the first organ to make its appearance. In Pl. XXXII, Fig. 25 *a*, which is drawn from a frontal section, an extremely early stage is shown at *pc.r.* That the rudiment is formed by the coming together of *free amoeboid cells of the blood* I believe there is no reason for doubting. At the stage represented in this figure, the similarity between many of the cells scattered freely about in the space between the ectoderm and endoderm and those which form this cell mass is perfectly apparent. There is certainly not the slightest evidence that the wall of the inner vesicle evaginates or its cells proliferate at this point; the line of demarcation between the two structures is distinct throughout, and shows no interruption in its continuity.

The rudiment does not long remain of one layer, but by the addition of other cells and by active cell division it soon becomes thicker and more compact (Fig. 25 *b*). The cell boundaries are gradually lost, and the solid mass is now firmly attached to the wall of the vesicle (Fig. 25 *c*). The rudiment, which has now an elongated form, is not in a horizontal position, but posteriorly is at a higher level than anteriorly.

When the shifting of the inner vesicle begins, the clump of cells is borne passively down towards the ventral side, but long before it has reached its definitive position a cavity has appeared in its center, around which the cells become arranged in an epithelium to form an elongated closed sac (Figs. 25 *d* and *e*).

The position of the rudiment at various stages during its descent has already been observed while considering the displacement of the inner vesicle from Pl. XXX, Figs. 9, 10, 11, and Pl. XXXI, Figs. 20, 24.

About the time that this change in position is accomplished the pericardial sac loses its attachment to the branchial wall, and grows considerably longer and wider. The cells composing the sac become very much flattened and attenuated, except in the dorsal wall, which is soon folded in longitudinally to form the heart in the usual way (Fig. 24, *d.w.pc.s.*). The pericardium in its definitive position is placed under the posterior floor of the pharynx, just to the right of the median line. It is not horizontal, *i.e.*, parallel with the surface of the stolon,

but the posterior is higher than the anterior end. This inclination is seen in Pl. XXIX, Figs. 4, 5, in which the pericardium is indicated at *pc*. In the latter figure, which shows about its final position, the pericardium is seen to extend from a point at a level nearly as high as the upper end of the stomach straight down to the stalk which connects the bud with the stolon.

*The Dorsal Tube and Ganglion.*

Of all the organs of the ascidian bud, that which has given rise to the greatest amount of discussion is the nervous system. Its origin and development have been matters of much dispute, and so wide is the difference of opinion concerning points of fundamental importance that there is little hope at present of harmonizing the conflicting statements of various authors.

A close relation between the dorsal tube and ganglion has been affirmed by many who hold to a common origin of the two, but is strenuously denied by others, who assert that the dorsal tube arises independently and has nothing whatever to do with the nervous system. Different authors have ascribed to these structures an ectodermal, a mesodermal, and an endodermal origin, and have thereby exhausted the entire series of possibilities.

Kowalewsky (12), for the buds of *Perophora*, was the first to describe an endodermal origin of the nervous system. According to him, the dorsal wall of the branchial sac evaginates to form a tube, which retains its connection with the branchial cavity, and which he calls the "Nervenrohr." In his later work on the budding of ascidians (13) he describes the rudiment of the nervous system in *Amaroucium* and *Didemnum* as "ein sehr langes, am vordern Ende ziemlich breites Rohr, dessen Lumen mit der Höhle des Kiemensackes zu communiciren scheint" (*l.c.*, p. 465). He did not follow the development of this tube, which he held to be derived from the endodermal vesicle, and was ignorant of its relation to the nervous system of the adult animal. It is probable, however, that he saw the ganglion in *Amaroucium*, at least, but failed to recognize it, for he says (*l.c.*, p. 465), "Bemerkenswerth ist noch, dass über dem Nerven-

rohr sich eine Anhäufung von sehr blassen Zellen befindet, welche bei weiterer Entwicklung zu verschwinden scheinen." *Amaroucium* is one of those ascidians in which the ganglion lies *above* the hypophyseal tube.

Ganin (5), who studied the bud development in *Didemnum* and *Botryllus*, derived the nervous system from a vesicle which he described as being cut off from the inner vesicle of the bud and converted into a long cylindrical tube lying over the dorsal wall of the branchial sac. The ganglion, according to Ganin, becomes differentiated from a part of this tube, the remainder of which forms a ciliated organ communicating with the branchial cavity. His description is very obscure, however, and the only points to be noticed are that the dorsal tube, according to this author, is derived from the endodermal vesicle and that it gives rise to the ganglion.

Giard (6) and Della Valle (3 and 4), who studied the bud development in different species of ascidians, contributed nothing of value concerning the nervous system, but both ascribe a common origin to the dorsal tube and ganglion.

The views of Seeliger (29) are very different from the foregoing. According to him, the dorsal tube and ganglion in the buds of *Clavelina* arise from a common rudiment, which is derived from mesodermal cells. This belief was not based on direct observation, since he did not examine sufficiently young stages, but was arrived at through theoretical considerations. The great similarity between the individual cells of the nerve-rudiment and the free blood cells in the body cavity of the bud Seeliger holds is good evidence for the mesodermal origin of this structure. He furthermore points out that the cells composing the ganglion of the larva would be carried off in the blood after the disintegration of that organ, and give rise in the bud to some of these free cells. The latter would, therefore, be "*directe Abkömmlinge eines frühere gangliösen Organs*," and it would be but natural for them to resume the function which they had once possessed. Van Beneden and Julin (33), on the contrary, in their work on the development of the buds of *Clavelina*, state that the nervous system is derived from the ectoderm, and first appears as a cord of cells lying close against

the ectodermal wall. Their description, however, is very incomplete and unsatisfactory.

Our more recent knowledge of the subject is due to the researches of Pizon (22), Oka (20), and Hjort (8) on *Botryllus*, of Salensky (27) on *Distaplia*, and of Caullery (1) on *Glossophorum* and *Diplosoma*. The first three authors, although they are in agreement concerning the origin of the dorsal tube, differ widely in respect to the derivation of the ganglion. According to all three, the dorsal tube in the *Botryllus* bud arises as an anteriorly directed evagination of the peribranchial cavity, ending blindly in front, but freely opening into the cavity at its posterior extremity. This tube grows forward, and its anterior end fuses with the wall of the branchial sac, whose cavity is then put into communication with the lumen of the tube, while the posterior connection becomes obliterated. The definitive opening of the hypophyseal tube is, therefore, secondary. So far we find these authors agreeing, but it is quite otherwise when we come to consider the origin of the ganglion.

Pizon maintains that the ganglion of the bud is derived directly from a fine nerve string, which grows out from the ganglion of the parent bud, or, in the first place, from that of the larva into the young bud. His view is not based on actual observation, and his arguments, which are far from satisfactory, fail to convince. He avers that a constriction of the ganglion from the wall of the dorsal tube does not take place, but, on the contrary, the figures of Hjort (8, *Botryllus*) prove fairly conclusively that such a constriction does actually occur. Hjort's contention that the ganglion is formed from the thickened ventral wall of the hypophyseal tube is based on a study of an unbroken series of stages and is clearly borne out by his figures. In a short note<sup>1</sup> on the budding of *Botryllus*, which was published recently, I added additional evidence in support of Hjort's view, and reproduced a drawing which showed beyond a doubt that the thickened ventral wall of the dorsal tube is pinched off to form the ganglion.

The account given by Oka is entirely different. According

<sup>1</sup> *Johns Hopkins University Circulars*, No. 119, June, 1895.



to this observer, cells wander out from the ectoderm, fasten themselves to the ventral wall of the dorsal tube, and there form the ganglion. These wandering ectodermal cells were also observed by Pizon, who described them as giving rise to a portion of the genital gland, to muscular fibers, and to certain cells of the blood. The principal difference, then, between the three authors is that, whereas Pizon and Oka hold to an independent origin of hypophysis and ganglion, Hjort maintains that there is a common rudiment for the two structures.

The results of Salensky (27) on the bud development of *Distaplia* do not stand in the slightest agreement with any of those obtained for *Botryllus*. The nervous system of the *Distaplia* bud is of ectodermal origin, according to him. Cells sink down at a very early period from the ectodermal wall and form a solid mass, which later acquires a cavity, increases in length, and produces a tube. The latter becomes differentiated into three parts; the anterior gives rise to the hypophysis, the middle to the ganglion, and the posterior portion to the visceral nerve. The hypophysis and ganglion have, therefore, a common origin. This mode of formation, however, is only true of the primordial bud; for all the other buds, which are produced from it by fission, derive their nervous system by division directly from that of the parent along with the rest of their organs.

It might be mentioned that in the buds of *Pyrosoma*, Salensky (26) has described a similar ectodermal origin of the nervous system, although Seeliger (30) in the same form derives the common rudiment of ganglion and hypophysis from mesodermal cells.

Salensky's results on *Distaplia* are directly contradicted by Hjort and Frl. Bonnevie (10). The latter find no trace of the nervous system in the early stage at which Salensky describes its first appearance, but maintain, on the contrary, that a forwardly directed diverticulum is later formed from the dorsal wall of the inner vesicle, just as in *Botryllus*, and that the ganglion is differentiated from the wall of the dorsal tube.

Pizon (22), in his work on *Botryllus*, states that he has made observations on the development of the dorsal tube in the buds of a number of other ascidians. In *Perophora* and *Clavelina* he



observed the tube over the dorsal wall of the branchial sac, but did not obtain stages which were young enough to enable him to determine its origin. He concludes, however, on the insufficient evidence of Kowalewsky's observations on *Perophora*, that the dorsal tube arises as a diverticulum of the endodermal wall. Since, in *Clavelina*, the later stages in the development of the dorsal tube are similar to those of *Perophora*, he holds that in this ascidian, also, the origin is the same. In two of the Polyclinidae, viz., *Amaroucium proliferum* and *Circinalium*, in *Didemnum niveum* and in *Astellium spongiforme*, he has observed the dorsal tube arising as an endodermal diverticulum, which acquires a secondary opening into the branchial sac at its anterior extremity, just as in *Botryllus*. In none of these forms did he determine the origin of the ganglion, but he comes to the unwarranted conclusion that this structure is derived, independently of the dorsal tube, in the same way as he has described for the Botryllidae.

Hjort (9) has recently studied the development of the neurohypophyseal system in the buds of *Glossophorum sabulosum*, one of the Polyclinidae, and Caullery (1) in *Glossophorum luteum*, *Circinalium concregens*, and *Diplosoma gelatinosum*, and, although both of these authors find that the dorsal tube arises in the manner described by Pizon, that is, as an anteriorly directed endodermal diverticulum, they give a different account of the origin of the ganglion. In all the species studied the ganglion is formed as a differentiation of the dorsal wall of the hypophyseal tube, and has, therefore, a common rudiment with the latter. Their results are in agreement with what Hjort has found in *Botryllus*, except that in all of the above-mentioned ascidians the hypophysis lies below the ganglion, whereas in *Botryllus* it is above.

Finally, Ritter (24), who has recently described the bud development of *Goodsiria*, a genus in which budding had not been observed before, finds a complete agreement, concerning the origin of the neuro-hypophyseal system, with Hjort's work on *Botryllus*.

In the same paper Ritter gives a preliminary account of some observations on the development of the buds of *Perophora*

*annectens* and *P. listeri*. In both species he derives the common rudiment of the hypophysis and ganglion from cells which wander out from the dorsal wall of the inner vesicle.

After this short review of the state of our knowledge regarding this much confused subject, I shall now give an account of my own observations on the development of the dorsal tube and ganglion in the buds of *Perophora viridis*.

My results, which are based on a study of an uninterrupted series of stages, have led me to believe that the conclusion which Seeliger drew from purely theoretical considerations concerning the origin of these structures in *Clavelina* is also true of *Perophora viridis*. I shall try to show that *the dorsal tube and ganglion are derived from amoeboid cells of the blood*.

The dorsal tube is formed long before the ganglion, and the rudiment from which it will arise is first indicated just after the collection of cells which is to produce the pericardium makes its appearance. When the difference in thickness between the right side and the rest of the vesicle is becoming apparent — hence at a time when the rotation is about beginning — in the anterior portion of the bud, a little to the left of the median dorsal line, there is seen an irregular elongated patch of cells very loosely grouped together and lying on the outer surface of the inner vesicle (Pl. XXX, Fig. 13, *d.t.r.*). The free amoeboid cells in the space between the ectoderm and endoderm are especially numerous in this region, and are closely associated with the collection of cells adhering to the vesicle. Although I have examined my sections with the greatest care under an oil-immersion lens, from the very first appearance of the rudiment, I have failed to find any indication of cell migration from the endodermal wall, and, therefore, cannot confirm Ritter's statement (*l.c.*, p. 368) that an "indistinguishable transition from the cells of the 'endoderm' to those of the neuro-hypophyseal anlage is to be traced," and also that cells can be found "in the act of migrating from the 'endoderm' into the anlage."

The line of separation between the rudiment and the wall of the vesicle is seen to be perfectly distinct and clearly marked,

and there is no evidence whatever of proliferation of endodermal cells at any point (Pl. XXXII, Fig. 26, *a*). During the early stages of development the cells which are to form the dorsal tube and many of the blood cells are absolutely identical in appearance and exhibit the same amoeboid character. So gradual is the transition from the free blood cells to the cells of the rudiment that it is at first impossible to say where the former end and the latter begin (Fig. 26, *b*).

I believe, therefore, that all the evidence shows that *the dorsal tube is derived from free amoeboid cells of the blood*.

By further additions from outside, and by active cell multiplication within the mass, the rudiment gradually increases in size; its cells become more closely packed together, and soon form an elongated solid cord, lying close against the dorsal wall of the vesicle in the anterior end of the bud (Fig. 26, *c*). Fig. 26, *a*, *b*, and *c*, illustrates the development up to this point; *a* is drawn from the same section as Pl. XXX, Fig. 13; *b* and *c* from the series to which Figs. 10 and 11 respectively belong. It has been stated above that the anterior portion of the inner vesicle is only slightly involved in the displacement already described; but that it is to a certain extent is proved by the fact that the rudiment of the dorsal tube first appears not exactly in the mid-dorsal line, but a little to the left of this (Fig. 13, *d.t.r.*). By the time the shifting of the vesicle has proceeded somewhat further than is shown in Fig. 11, the string of cells, which is now solid, has been carried up to the median plane.

Very shortly after it has reached its definitive position, a lumen appears in the center of the rudiment throughout its entire length, and around this the cells become arranged into a one-layered epithelium (Pl. XXXII, Fig. 26, *d* and *e*).

By following the course of development up to this point, it is seen how an epithelial tube does actually arise from free mesenchymatous cells, — a thing which Hjort has characterized as most improbable. In criticising Seeliger's view of the origin of the neuro-hypophyseal system in *Clavelina*, this author says (8: p. 602): "Die Wahrscheinlichkeit dafür, dass ein Ganglion und ein epitheliales Rohr sich aus zusammengehäuften

Mesodermzellen bilden sollte, scheint mir so gering zu sein, wie für die Auffassung Herdman's, dass die innere Blase der Knospenanlage einen solchen Ursprung habe."

About the time that the peribranchial cavity is completely cut off from the inner vesicle, the anterior extremity of the dorsal tube fuses with the dorsal wall of the branchial sac, an opening breaks through, and the lumen of the tube is put into communication with the branchial cavity. The posterior end of the tube abuts against the anterior wall of the cloaca, but never opens into the latter, in contrast with the condition found in *Botryllus* and many other ascidians.

Pl. XXX, Fig. 14, represents a median sagittal section of a bud before the complete separation of the peribranchial cavity, and, therefore, before the dorsal tube has acquired an opening into the branchial sac. The section passes through the entire length of the tube (*d.t.*), which is seen to be closed at both ends and made up of an epithelium of one layer.

In my preliminary work on the budding of *Perophora*, already referred to, I made the statement that "the ganglion is formed by a thickening of the *dorsal* wall of the tube, which eventually becomes constricted off in the manner described by Hjort for *Botryllus*, although in the latter it is the *ventral* wall of the tube which gives rise to the ganglion." More careful study of very young stages, however, has convinced me that the above is not an accurate description of the formation of the ganglion.

After the communication between the dorsal tube and branchial sac has been established, a few cells, identical in appearance with the amoeboid blood cells, are found adhering to the dorsal surface of the tube throughout the greater part of its length; this elongated, loose patch of cells constitutes the rudiment of the ganglion (Pl. XXXII, Fig. 27, *a*, *gl.r.*).

It is a difficult question to decide whether these cells are entirely cells of the blood, as their appearance indicates, or whether they are derived by proliferation in the wall of the tube, for in many places the boundary line of the latter is broken, and there is no sharp demarcation between the cells of the rudiment and those of the tube, as seen in Fig. 27, *a*. Many sections, however, such as the one shown in Fig. 27, *b*,



which represents a slightly older stage, leave little room for doubt that nuclei do wander out into the rudiment. But, on the other hand, I think that this figure and Fig. 27, *c*, show equally well that blood cells are added to the mass from the outside.

I have, therefore, come to the conclusion that *the ganglion has a double origin, and that the wall of the tube and free amoeboid cells coöperate in forming it. But, as we have seen that the dorsal tube is made up of cells of the blood, it is, therefore, to be remembered that the ganglion is ultimately derived solely from this source.*

The ganglionic rudiment is at first a very irregular heap of cells, and is closely associated at the periphery with surrounding blood cells. The cell boundaries are completely lost very early, and the mass rapidly increases in size by multiplication of nuclei within, by further acquisition of cells from without, and by continued migration of nuclei from the wall of the tube (Fig. 27, *c*). The nuclei now arrange themselves in a couple of layers around a central core, in which fine fibrils are laid down, and the ganglion becomes completely marked off from the wall of the tube; the definitive structure is now attained. Fig. 27, *d* and *e*, illustrates the later course of development.

My observations, therefore, have led me to believe that the hypophyseal tube and the ganglion are formed only in part from a common rudiment, and in this respect to take a middle ground between Hjort, Salensky, and Caullery, on the one hand, who have described a common origin for these structures in the ascidians studied by them, and Oka and Pizon, on the other, who maintain that they arise independently. In deriving the hypothesis and ganglion, however, from the cells of the blood, I differ widely from all previous observers, with the exception of Seeliger. Concerning the origin of the dorsal tube in the buds of *Perophora*, my results are totally opposed to the conclusion of Pizon, which, as stated above, is not based on sufficient evidence; namely, that "le tube dorsal des Pérophores a la même évolution que le tube dorsal des Botryllidés" (*l.c.*, p. 130). A study of the younger stages would have convinced him of his error. *Perophora viridis*, at all events, presents an exception to the general rule laid down by Pizon that "Chez toutes ces



familles d'Ascidies composées (Clavelinidae, Perophoridae, Botryllidae, Polyclinidae, Distomidae, Didemnidae, et Diplosomidae) l'organe vibratile débute par un tube aveugle, formé par un diverticule de la vésicule endodermique primitive" (*l.c.*, p. 131).

### *The Sexual Organs.*

My observations on the development of the sexual organs have not been carried beyond quite an early stage, but, so far as they go, they closely agree with the description given by Van Beneden and Julin (33) for the buds of *Perophora listeri*.

Shortly after the peribranchial cavity has been completely divided off from the branchial sac, a small collection of cells appears between the two arms of the U-shaped digestive tract, and at the level of the duct of the "organe réfringent," almost at the point where the latter is connected with the stomach. The sexual organs arise from this little spherical mass of cells, which are at first but loosely held together and identical in appearance with the amoeboid cells of the blood. Pl. XXXII, Fig. 32, *a*, which is precisely similar to Van Beneden and Julin's Pl. XVI, Fig. 5, *b*, shows the intimate relation between the cells of the blood and those of the rudiment (*g.r.*), so that there can be no doubt that the two are identical. The connection, already described by the Belgian authors, of some of the peripheral cells of the mass with surrounding blood cells by protoplasmic processes is distinctly seen in the figure at *m.c.* A small, irregular cavity (*c.g.r.*) is also shown in the center of the clump of cells. Some of the free cells lying above the rudiment (Fig. 32, *a*, *g.c.*) are seen to be spindle shaped; these soon become joined, end to end, to form a solid cord, united at one end to the spherical mass of cells and taking a course parallel to that of the intestine (Fig. 32, *b*, *g.c.*). This figure represents a later stage, in which the cavity is considerably enlarged and the genital cord (*g.c.*) is present as a solid single row of cells (*cf.* Pl. XII, Fig. 2, of Van Beneden and Julin).

A furrow, which appears opposite to the attachment of the cord, now divides the hollow sphere into two lobes, the cavities of which are not completely separated, but remain in communi-

cation for a time. In Fig. 32, *c*, the division of the originally simple sphere is seen, but the section is not in the proper plane to show the connection between the two cavities.

One of these lobes gives rise to the testis, the other to the ovary, according to Van Beneden and Julin, who have described in detail how, from the primitive, simple sphere and the single cord of cells, testis, ovary, vas deferens, and oviduct are all differentiated.

My observations would, therefore, seem to support the view of the Belgian authors that the male and female sexual organs do not arise from separate vesicles, as described by Kowalewsky (12) in the buds of *Perophora*, but are formed from one and the same rudiment.

#### *Summary of Results.*

(1) The rudiment of the *Perophora* bud, like that of all other ascidians, consists of two vesicles, an outer and an inner one. The former is derived from the ectoderm of the stolon, the latter from the thickened evaginated wall of the stolon partition.

(2) At an early stage the right side of the inner vesicle is found to be much thicker than the remaining portion, and, by a peculiar process of rotatory growth or displacement of the vesicle, is carried down to the ventral side of the bud, where it forms the floor of the pharynx. This process seems to be due to the growth and flattening out of the cells composing the whole wall of the vesicle, except in the thickened region. I am at a loss to explain why the displacement of the vesicle should occur, and to discover the phylogenetic significance of it, if it have any.

(3) The peribranchial sacs arise asymmetrically. As the displacement proceeds, the wall of the inner vesicle is folded in at the point where the right side of the vesicle joins the stolon partition to form the *left peribranchial sac*. The connection of the latter with the partition of the stolon is retained until a much later stage. A longitudinal furrow appearing high up on the right side of the inner vesicle separates off the right

peribranchial sac, and is gradually borne ventrally, as the shifting of the vesicle continues. The constriction of the whole peribranchial cavity eventually takes place in such a way as to cut off from the peribranchial sac a saddle-shaped bag, composed of the median dorsal connecting piece or cloaca and the two lateral portions of the cavity. Anterior and posterior extensions of the latter grow out and surround respectively the anterior and posterior ends of the pharynx. The posterior prolongations are possibly to be regarded as homologous with the epicardial sacs of some other ascidians.

(4) The endostyle appears early as a longitudinal groove in the middle of the thickened portion of the vesicle; from its primitive position on the right side it is moved down to the ventral mid-line by the displacement of the vesicle.

(5) The digestive tract grows out laterally as a blind tube from the posterior end of the inner vesicle high up on the left side. During the change in position of the vesicle its opening into the latter is carried up into the median plane.

The "organe réfringent" arises as a tubular diverticulum from the anterior face of the stomach, and produces a dichotomously branched system of tubules, which surround the intestine and terminate in little dilated vesicles or ampullae.

(6) All the evidence goes to show that the pericardium is formed from free amoeboid cells of the blood. It first appears as a clump of cells adhering to the outer surface of the inner vesicle far up on the right side, and through the shifting of the vesicle is brought down to the ventral side.

(7) The dorsal tube and ganglion are formed only in part from a common rudiment, but there is every reason to believe that both are derived solely from cells of the blood. The former appears as an elongated, solid mass of cells, which lies close against the outer surface of the inner vesicle, a little to the left of the median dorsal line. When the displacement of the vesicle is completed, the rudiment lies in the median plane; it then acquires a lumen, which is put into communication anteriorly with the cavity of the pharynx. The ganglion has a double origin, and is formed by proliferation of the upper wall of the dorsal tube and also by addition of cells of the blood.

(8) The sexual organs have a common origin from free amoeboid blood cells, both testis and ovary arising from one and the same spherical rudiment.

### *Concluding Remarks.*

The results which I have obtained from the study of the budding of *Perophora viridis* furnish additional evidence in support of the view that the development of the bud and that of the embryo do not proceed along parallel lines. The attempts which have been made to harmonize the facts of budding with the germ layer theory have been totally futile in the case of ascidians, and any hypothesis which explains budding in the ascidians as a process of regeneration, by which the organs of the parent, or their germ layers, give rise to similar organs in the bud, must, in the light of known facts, be ruled out.

The rudiment of the bud in all groups of compound ascidians is composed of two vesicles, one within the other, enclosing between them free cells of the blood. The outer vesicle is always derived from the ectoderm of the parent, and gives rise to the ectodermal covering of the bud. The origin of the inner vesicle, however, is not the same in all ascidians. In the Botryllidae it arises, in both embryozooid and blastozooid, from the peribranchial wall, which is formed in the first place from the ectoderm of the embryo. This vesicle is, therefore, ultimately of ectodermal origin in Botryllus, whereas in all other ascidians it comes from an endodermal structure of the parent. Although derived in the two cases from different germ-layers, the inner vesicle may go to form the same organs in the bud but organs which are of widely different origin in the larva; for example, the digestive tract and nervous system (Hjort, Botryllus (8), Distaplia (10), Glossophorum (9)). It is, therefore, perfectly evident that the fate of the inner vesicle entirely precludes the application of the "germ-layer theory" to the ascidian bud.

In *Perophora viridis* the important part played in the bud development by cells of the blood only increases the disagreement. Organs which in other ascidian buds arise from either ectoderm or endoderm are here formed by the coming together



of free amoeboid cells in the body cavity. Such is the origin, for instance, of the nervous system and pericardium, which, however, in the embryo are respectively ectodermal and endodermal structures.

Since in the development of the bud and that of the larva the same end is reached by entirely different roads, and in the former organs do not proceed from corresponding larval organs or even their germ-layers, and, moreover, since in the bud a rudiment derived from one and the same embryonic germ-layer may give rise to structures of widely different nature, one is compelled to believe with Hjort that "die Knospung der zusammen gesetzten Ascidien ein Entwicklungsprocess ist, in welchem sämtliche Organe durch 'Neubildung' aus einer sehr primitiven Anlage entstehen." . . . "Die Knospe muss ihre eigenen Gesetze haben und muss, da sie aus einer wesentlich anderen Anlage hervorgeht als die Larve, auf andere Weise gebildet werden. Ebenso wie das Ei, muss das Material, die Anlage, welche den Ausgangspunkt für die Entwicklung bildet, als ganz undifferenziert gedacht werden und muss alles enthalten, was zur Bildung eines ganzen Individuums nötig ist, ebenso wie die Blastula des Eies" (9; p. 225).

The behavior of the blood cells in the bud development of *Perophora viridis* is of much interest, owing to the number and variety of organs in whose formation they are concerned. As we have seen, these cells give rise to the pericardium, the dorsal tube, the ganglion, and the sexual organs; they also produce the musculature, and, as Kowalewsky (14) and Seeliger (32) have shown in other ascidians, in *Perophora* also they doubtless become the cells of the cellulose test.

As I have repeatedly emphasized in describing the formation of the various organs in question, there is not the slightest discoverable evidence that cells are given off from the wall of the vesicle at the places where such organs arise, or at any other point, for that matter, and the similarity between the cells which make up the different rudiments and the free amoeboid cells of the blood is so perfectly apparent, especially at early stages, that the two are certainly identical.

The origin of these blood cells must, however, be left in



doubt, as it would, of course, be impossible to prove that all or any of them are direct descendants of the embryonic mesoderm, and the most diligent search fails to show that the inner vesicle of the bud is the source from which they are derived. Even though it cannot be shown to be the case, however, it is quite possible that at certain times the primitive vesicle does give off cells which are set free in the blood, and, as this is an undifferentiated structure and plays such an important rôle in the development, the supposition is probable. But, whatever be their origin — whether it is the mesoderm of the embryo or the inner vesicle of the bud-rudiment or both — amoeboid cells floating freely about in the blood spaces become aggregated at certain places and form pericardium, dorsal tube, ganglion, and sexual organs.

A discussion of the nature of these cells and the part played by them in the developing bud will, however, not be out of place. In the case of a fixed organ, like the ganglion or pericardium, which has a definite and determined position, the cells destined to form it must, by virtue of their motile power, come together at the right time and place. Weismann (35; pp. 161, 162), in discussing the process of gemmation in *Clavelina* as described by Seeliger (29), supposes that "these cells contain very different kinds of idioplasm; one, for instance, might contain 'muscle determinants,' and another, 'nerve determinants,' and a third, 'blood corpuscle determinants.'" He further adds that "until we know more of the actual facts concerned, we can only — however unsatisfactory such an assumption may be — attribute to the cells a tendency to become attached at definite points according to the manner in which they have previously been determined."

It seems to me, however, that the opposite assumption, which Weismann regards as less likely, namely, "that these cells develop into muscle, nerve, or sexual cells, according to their point of attachment," is more in accordance with the facts presented by the bud development of *Perophora viridis*.

We have already seen in the very young bud, when it consists merely of two simple layers, and before there is the slightest indication of the appearance of any organs, that cells

are attached in many places to the inner surface of the ectoderm and outer surface of the endoderm, but that they are not more numerous at any one spot than another (Pl. XXX, Figs. 7, 8). Their power of amoeboid movement over any surface with which they come in contact would account for their presence on the walls of the vesicles.

It would seem more probable that these cells are all alike and indifferent, and that the nature of the organs to which they give rise is determined, not by any prearranged condition of their idioplasm, but by the particular point to which they happen to become attached. I regard it as a significant fact that cells are found, not only at the places where organs will arise, but also at many other points. Those of the cells which chance to fall, as it were, on fertile soil will undergo further development, and, under the formative influence exerted upon them by that portion of the wall to which they adhere, will be utilized in building up a definite structure.

All parts of the walls cannot possess a specific determining power, and such cells as lodge on barren ground are not further modified, and do not furnish material for the formation of organs.

According to this view, one of these cells is the equivalent of any other, and it is only a few that find favorable positions and have their latent possibilities called forth. Those which become attached at a point high up on the right side of the inner vesicle in the posterior region of the bud will form the pericardium; others on the dorsal side, at the anterior end, will give rise to the dorsal tube, and still others, which lodge on the upper wall of the latter, will help to construct the ganglion; some adhere to the inner surface of the ectoderm, lengthen out, and become muscle fibers; some wander through the ectoderm, and on the external surface are transformed into the cells of the cellulose test; while others find a definite place in the posterior region of the bud and develop into the sexual organs.

This view is opposed to the supposition of Seeliger spoken of above, that in *Clavelina* the ganglion of the bud is formed from free cells of the blood which had earlier composed the larval ganglion and been liberated on the dissolution of that

organ; these cells would, therefore, have already possessed a ganglionic nature, and would merely resume in the bud their former function.

On any such assumption, it is almost impossible to imagine how isolated specific cells, moving freely about in the blood, could reach their proper destination. On the reverse assumption, however, the presence of these cells at any particular point is accidental, but, once there, their potentialities are called out under the specific formative influence of the place of attachment.

BALTIMORE, MD.,  
April 18, 1896.

#### APPENDIX.

Since the foregoing was written an article by Prof. W. E. Ritter has appeared in this journal (Vol. XII, No. 1) on the budding of *Goodsiria* and *Perophora*. Of the interesting account given of the budding in the former genus I have nothing to say, but, as the results which he has obtained from his study of the process in *Perophora* differ in some particulars from my own on the same genus, I desire to add another word.

After reading his paper I again examined my sections with exceptional care under an oil-immersion lens, but am more firmly convinced than ever that the origin of the pericardium and dorsal tube and ganglion, points on which Ritter and I disagree, is that which I have described above; and that these structures in *Perophora viridis*, at all events, do arise *solely* from free amoeboid cells of the blood. I am confident that in the form I have studied no cells are given off *directly* from the wall of the inner vesicle to the rudiments of the organs in question, as no cell proliferation can be observed, and never is there the slightest interruption in the boundary line of the wall.

Although the peculiar rotation or transverse shifting of the inner vesicle, which I have described as occurring in *Perophora viridis*, does not take place to as great an extent in *P. annectens*, it nevertheless is found, and in this respect Ritter has fully confirmed my observations. Some time before the appearance

of his completed paper he published a preliminary note,<sup>1</sup> in which he stated that "when the differentiation of the 'endoderm' into the branchial and two peribranchial sacs takes place, it does so in such a way that the developing blastozoöid is connected with the double-walled partition of the stolon, not by the *branchial sac*, as has been hitherto supposed, but by the left peribranchial sac"; but no mention was made of the rotation of the vesicle by means of which this condition is brought about.

Shortly afterwards, a brief account<sup>2</sup> of my results on *P. viridis* was published, in which I described the rotation of the inner vesicle and showed by means of figures that this process causes the septum of the stolon to become connected with the left peribranchial and not the branchial sac. I have repeated this statement, because from the account given by Ritter of the relation of the bud to the stolon (*loc. cit.*, pp. 190, 192) one would infer that all my observations were merely a confirmation of his, as he says, "Lefevre fully confirms my results in this particular"; whereas, before the appearance of my note, the above quotation, which affords no explanation of the process which brings about the connection of the septum with the left peribranchial sac, was the only published statement of his on the subject.

I am fully aware of the danger of inferring that, because something takes place in a certain way in the bud development of one ascidian, the same is true of another, however closely the two forms may be related, and in the light of all we know concerning the budding of different ascidians it is not surprising to find even great differences. It is, therefore, perfectly possible that both Ritter and myself are correct in our observations on the derivation of the pericardium and dorsal tube in the two species of *Perophora* which we have studied; and that in the one these organs are formed both by cells given off *directly* from the wall of the vesicle to their rudiments, and also by free cells of the blood, while in the other it is from this latter source alone that they arise. Ritter's account, however, is not abso-

<sup>1</sup> *Anat. Anz.*, Bd. x, No. 11.

<sup>2</sup> *Johns Hopkins University Circulars*, No. 119, June, 1895.



lutely convincing to my mind, and, in fact, he himself states (*loc. cit.*, p. 197) that his "conclusion rests upon a *preponderance of evidence*," as there is certainly evidence which is "indicative of a mesenchymal origin of the cells." I do not consider as valid his argument that the connection between the wall of the inner vesicle and the pericardial rudiment is too strong to be a mere contact, and cannot be supposed to be a secondary one, as a complete separation takes place later. A glance at Pl. XXXII, Figs. 25, *a*, *b*, and 26, *a*, *b*, of this paper will show that, even granting for the moment that the cells of these two rudiments have been given off from the vesicle, they are very loosely attached at this early stage to the wall, and that, however intimate the connection between the two may become later, it must necessarily be a secondary one. In *P. viridis* I believe it to be merely a contact, although a firm one, and as the pericardial sac ultimately breaks away from the vesicle, on *a priori* grounds alone one would not expect to find an organic union; I have the additional evidence that I have been unable to discover any interruption in the boundary line of the vesicle, there being everywhere a sharp demarcation between the wall and the rudiment. Any argument from this source must, therefore, be ruled out, for it is certainly true that a firm secondary contact is not only possible, but actually does occur in *P. viridis*.

It is to be regretted that Ritter did not observe earlier stages in the development of the pericardium and dorsal tube, but his Figs. 68 and 74, and the statement on page 197 in regard to the pericardium, that he has found "no sections in which *at some focuses* I cannot see the separating line to be interrupted," strongly incline one to accept his conclusions for *P. annectens*.

I share the belief with Ritter that there is very good ground for holding that the inner vesicle gives off cells into the body space, and is, therefore, one source, at all events, of mesenchyme cells, which are found free in the blood, and apparently take part in the formation of the pericardium and dorsal tube. It is hardly possible to suppose, as he states, that these cells are all derived from the mesenchyme of the embryo, and, in fact, the inner vesicle of the buds is about the only place where one could look for their origin. I do not consider, however, that



the Figs. 77, 78, which he gives in support of his statement that he believes he has observed instances where this is taking place, are cases in point; it does not seem to me that there is any evidence that the cells marked *b.c'* have come from the vesicle, as there is no connection between the two, and they might simply be outside cells lying in depressions of the wall.

I have observed no cases in *P. viridis* where I could be positively sure that cells of the blood were derived in this way, but I regard it as extremely probable that at certain times or in certain buds at least such a process does occur, for it is difficult to imagine where these cells come from if not from the inner vesicle. If this be true, then the pericardium and dorsal tube in *P. viridis* are ultimately derived from the vesicle, and in *P. annectens* those cells which are directly given off into the rudiments of these structures and the blood cells which aid in their formation are cells of the same kind, having come in both cases from the wall of the vesicle of the same bud or a sister bud.

I have recently had the opportunity of studying the bud development in another genus of the Clavelinidae, namely, *Ecteinascidia*, Herdman, and in this form I have observed undoubted instances where cells are passing out from the vesicle to be set free in the blood; they are simply budded off into the body space, and the appearance is quite different from that of Ritter's Figs. 77, 78. A brief account of the development will shortly appear in the *Anatomischer Anzeiger*, but I may state here that I have found the dorsal tube, pericardium, and sexual organs to be all formed in large part by cells which are given off *directly* from the wall of the inner vesicle to their rudiments, but which are unquestionably supplemented by amoeboid cells from without. Here, therefore, the two processes do occur without a doubt, but, as all the cells which are concerned in the formation of these organs are derived from the same source — some, however, only indirectly — there is no essential difference between them.

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## DESCRIPTION OF THE PLATES.

All the drawings have been made with a camera lucida. The lenses used were those of Zeiss, and the magnification is given after the description of each figure.

## REFERENCE LETTERS.

<i>a.</i>	anus.	<i>int.</i>	intestine.
<i>amp.</i>	ampulla.	<i>in.v.</i>	inner or primitive vesicle.
<i>bl.s.</i>	blood sinus of stolon.	<i>l.a.ex.</i>	anterior extension or pouch of left peribranchial sac.
<i>br.o.</i>	branchial orifice.	<i>l.d.t.</i>	lumen of dorsal tube.
<i>br.s.</i>	branchial sac.	<i>l.p.ex.</i>	posterior extension or pouch of left peribranchial sac (left epicardial sac?).
<i>br.w.</i>	branchial wall.	<i>l.pb.s.</i>	left peribranchial sac.
<i>c.g.r.</i>	cavity of rudiment of sexual organs.	<i>m.c.</i>	free amoeboid cells of blood.
<i>cl.</i>	cloaca or atrium.	<i>ml.c.</i>	muscle-cells.
<i>cl.o.</i>	cloacal orifice.	<i>o.e.</i>	oesophagus.
<i>c.p.c.r.</i>	cavity of pericardial rudiment.	<i>o.r.</i>	"organe réfringent" or pyloric gland.
<i>c.pt.</i>	cavity of stolon double-walled partition.	<i>o.r.d.</i>	duct of "organe réfringent."
<i>c.t.</i>	cells of cellulose test.	<i>o.r.r.</i>	rudiment of "organe réfringent."
<i>d.t.</i>	dorsal tube or hypophysis.	<i>p.b.w.</i>	peribranchial wall.
<i>d.t.r.</i>	rudiment of dorsal tube.	<i>p.c.</i>	pericardium.
<i>d.w.p.c.s.</i>	thickened dorsal wall of peribranchial sac, which invaginates to form the heart.	<i>p.c.r.</i>	pericardial rudiment.
<i>e.br.o.</i>	ectodermal invagination to form the branchial orifice.	<i>p.t.</i>	double-walled partition or cloison of stolon.
<i>ec.</i>	ectoderm.	<i>r.a.ex.</i>	anterior extension or pouch of right peribranchial sac.
<i>e.cl.o.</i>	ectodermal invagination to form the cloacal orifice.	<i>r.p.ex.</i>	posterior extension or pouch of right peribranchial sac (right epicardial sac?).
<i>end.</i>	endostyle.	<i>r.pb.s.</i>	right peribranchial sac.
<i>f.l.pb.s.</i>	fold which forms left peribranchial sac.	<i>r.st.c.</i>	remnant of connection between the stolon partition and left peribranchial sac.
<i>f.r.pb.s.</i>	fold which forms right peribranchial sac.	<i>r.w.in.v.</i>	thickened wall of inner vesicle on right side.
<i>g.c.</i>	genital cord.	<i>st.</i>	stomach.
<i>gl.</i>	ganglion.	<i>st.c.</i>	connection of stolon partition with inner vesicle or with left peribranchial sac.
<i>gl.r.</i>	rudiment of ganglion.	<i>st.w.</i>	wall of stomach on anterior side.
<i>g.r.</i>	rudiment of sexual organs.	<i>stl.</i>	stolon.
<i>g.s.</i>	branchial stigmata or gill slits.	<i>t.</i>	cellulose test.
<i>g.s.r.</i>	rudiment of branchial stigmata.		
<i>gt.</i>	digestive tract.		
<i>gt.r.</i>	rudiment of digestive tract.		
<i>ht.r.</i>	rudiment of heart.		





## EXPLANATION OF PLATE XXIX.

All the figures of this plate were drawn from specimens mounted as whole objects. The test is only represented in Fig. 1. The buds were stained slightly in borax carmine, and were perfectly transparent.

FIG. 1. Shows evagination of ectoderm and partition of stolon to form the bud-rudiment.  $\times 170$ .

FIG. 2. Slightly older rudiment, seen from right side.  $\times 170$ .

FIG. 3. Still older rudiment, seen from right side, and showing anterior elongation and constriction of the stolon connection. Rudiment of dorsal tube is shown; that of pericardium is present, but is not drawn in the figure.  $\times 170$ .

FIG. 4. Young bud, seen from right side, at a stage when development of peribranchial cavity, pericardium, and digestive tract is well advanced.  $\times 170$ .

FIG. 5. Bud considerably older than the last, showing extensions of peribranchial sacs, formation of branchial stigmata, and further development of digestive tract, pericardium, etc.  $\times 170$ .





Fig. 1.

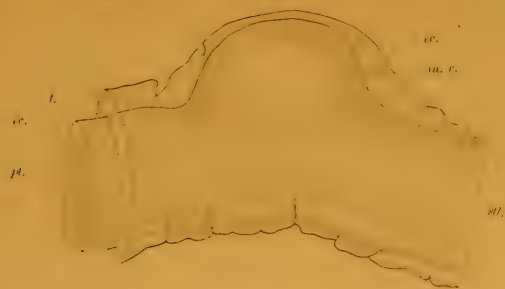


Fig. 2.



Fig. 3.

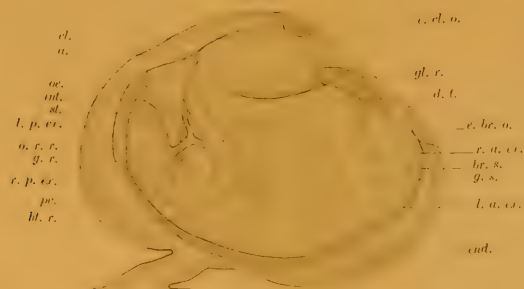
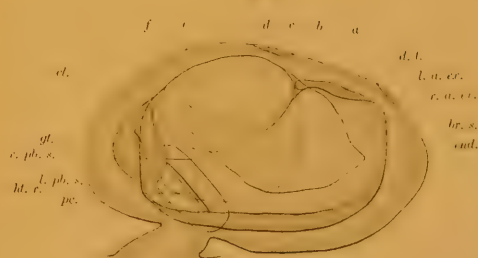


Fig. 3.



Fig. 4.









## EXPLANATION OF PLATE XXX.

The test is only shown in Figs. 6 and 7, and in Figs. 9-15 the ectoderm is represented merely by a line. The elements of the blood have not been drawn in Figs. 10-15.

FIG. 6. Transverse section of stolon, showing double-walled partition, *pt.*  $\times 500$ .

FIG. 7. Transverse section of stolon at point where a bud is beginning to form. The ectoderm on one side is thickened, and the double-walled partition is much thickened and dilated.  $\times 500$ .

FIG. 8. Transverse section through a very young bud-rudiment and stolon, at about the stage of Fig. 1, Pl. XXIX. The walls of the partition are coming together to close off the inner vesicle, *in.v.* Blood cells are found lying against the outer surface of the latter.  $\times 500$ .

FIG. 9. Transverse section through posterior end of a bud-rudiment at about the stage of Fig. 3, Pl. XXIX, showing thick wall of inner vesicle on right side, *r.w.in.v.*, and beginning of pericardial rudiment, *pc.r.*  $\times 500$ .

FIG. 10. Transverse section through posterior end of a young bud, showing an early stage in the displacement or shifting of the inner vesicle. The wall of the latter is being bent in at the point indicated by the line *a*.  $\times 200$ .

FIG. 11. Transverse section through posterior end of bud slightly older than last, showing further progress of the displacement of the vesicle. The pericardial rudiment, *pc.r.*, is at a much lower level than in the last figure, and the wall of inner vesicle is being bent in further at *a*; this is the fold which will form the left peribranchial sac, *f.l.pb.s.*  $\times 200$ .

FIG. 12. Transverse section through the middle region of same bud as the one shown in Fig. 11. The section lies in front of the connection with the stolon partition, and shows the beginning of the fold which will form the right peribranchial sac, *f.r.pb.s.* The rudiment of dorsal tube, *d.t.r.*, is also shown.  $\times 200$ .

FIG. 13. Transverse section through the anterior end of a bud slightly older than Fig. 9. The rudiment of the dorsal tube is shown, and consists at this stage of a few scattered cells, adhering to the wall of the inner vesicle a little to the left of the median dorsal line, *d.t.r.* The difference in thickness between the right side and the rest of the vesicle is seen to be but slight in this region.  $\times 200$ .

FIG. 14. Median sagittal section of a bud at about the stage of Fig. 4, Pl. XXIX, showing the position and extent of the dorsal tube, *d.t.* The peribranchial cavity is not yet completely constricted off from the branchial sac.  $\times 200$ .

FIG. 15. Transverse section through the extreme posterior end of a bud, showing the origin of the gut, *gt.r.*, as a diverticulum on the left side of the branchial sac.  $\times 200$ .





Fig. 6.



Fig. 7.



Fig. 10.

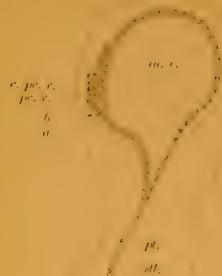


Fig. 11.

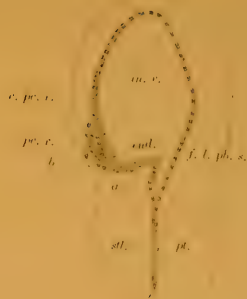


Fig. 8.

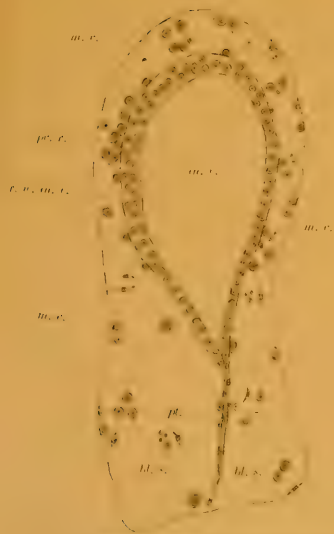


Fig. 9.



Fig. 12.

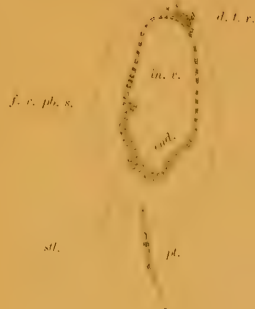


Fig. 13.

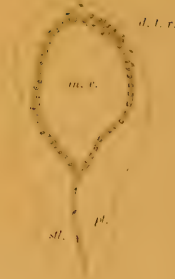


Fig. 15.

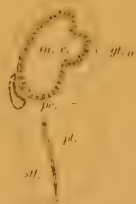
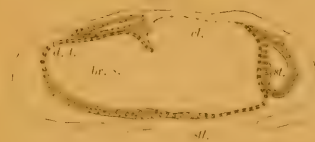


Fig. 14.









## EXPLANATION OF PLATE XXXI.

In all the figures of this plate the ectoderm is represented by a line, and the elements of the blood have been omitted for the sake of simplicity.

FIGS. 16-21. Series of transverse sections of a bud at about the same stage as that shown in Fig. 4, Pl. XXIX. The sections are taken respectively at the levels indicated by the lines *a*, *b*, *c*, *d*, *e*, and *f*, in Fig. 4. Fig. 16, line *a*, the most anterior section, shows undivided portion of branchial sac, *br.s.*, and dorsal tube, *d.t.* Fig. 17, line *b*, is a little further back, and shows anterior extension of right peribranchial sac, *r.a.ex.* Fig. 18, line *c*, is taken immediately in front of the cloacal cavity, and shows the extensions of the peribranchial cavity on both sides. The section passes through the extreme posterior end of the dorsal tube, *d.t.* Fig. 19, line *d*, passes through the anterior end of the cloacal cavity, *cl.*, which is seen to connect the lateral portions of the peribranchial cavity. Fig. 20, line *e*, is taken from the posterior end of the bud, and shows the connection of the left peribranchial sac with the stolon partition at *st.c.* The pericardial sac, *pc.*, is also shown in this region. Fig. 21, line *f*, shows the undivided extreme posterior end of the branchial sac. The section passes through a portion of the intestine, *int.*, and behind the connection with the stolon partition.  $\times 200$ .

FIGS. 22-24. Series of transverse sections of a bud of about the same age as the one represented in Fig. 5, Pl. XXIX. Fig. 22 is a section through the anterior region, and shows the extensions of the peribranchial sacs, *r.a.ex.* and *l.a.ex.* Fig. 23 is drawn from a section which is taken from the middle region of the bud, and shows the median portion or cloaca, *cl.*, connecting the lateral divisions of the peribranchial cavity, *r.pb.s.* and *l.pb.s.*, which is now entirely cut off from the branchial sac, *br.s.* Fig. 24 shows the posterior extensions (epicardial sacs?) of the peribranchial cavity, *r.p.ex.* and *l.p.ex.*, and the remnant of the connection with the stolon partition, *r.st.c.*  $\times 200$ .







Fig. 16.



Fig. 17.

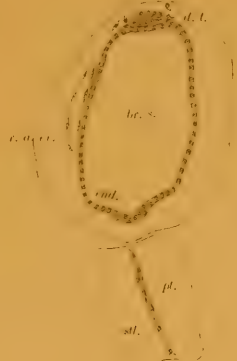


Fig. 18.



Fig. 19.

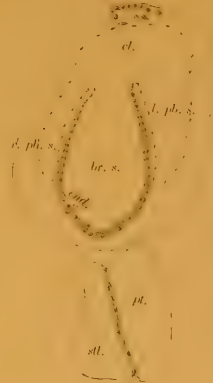


Fig. 20.

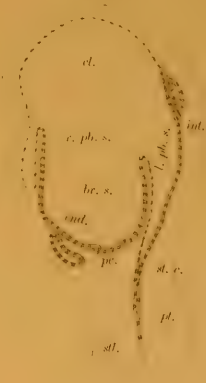


Fig. 21.

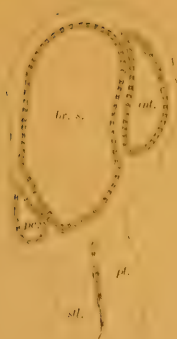


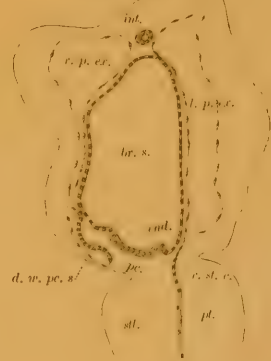
Fig. 22.



Fig. 23.



Fig. 24.







## EXPLANATION OF PLATE XXXII.

FIG. 25, *a, b, c, d*, and *e*. Sections illustrating the development of the pericardium. *a* is from a frontal section at a very young stage, and shows the rudiment when it consists merely of a few cells loosely attached to the inner vesicle. The remaining figures are drawn from transverse sections. In *b* and *c* the rudiment has increased in size; cells are being added from without, and nuclei within the mass are seen to be dividing. In *d* and *e* a cavity, *c.p.c.r.*, has appeared in the center of the rudiment, which is now much larger, and the cells are becoming arranged into a one-layered epithelium.  $\times 700$ .

FIG. 26, *a, b, c, d*, and *e*. Transverse sections representing stages in the development of the dorsal tube. In *a* a few cells are seen loosely grouped together, and lying on the wall of the inner vesicle. In *b* the association with surrounding blood cells is apparent. In *c* and *d* the mass has become compact, and cell-boundaries have entirely disappeared; in the latter figure the lumen in the center has begun to form, *l.d.t.* In *e* the tube is completely formed, and the cells are arranged around the lumen in a one-layered epithelium.  $\times 700$ .

FIG. 27, *a, b, c, d*, and *e*. Transverse section showing the development of the ganglion. In *a* and *b* a few cells resembling amoeboid cells of the blood are seen lying on the dorsal side of the dorsal tube, *d.t.* The outer membrane of the latter is broken at this point, and nuclei appear to be wandering out into the rudiment, *g.l.r.* In *c* and *d* the rudiment is much enlarged, and in the latter the nuclei are beginning to arrange themselves peripherally. In *e* the wall of the dorsal tube under the ganglion has been re-formed, and the central portion of the latter is now free from nuclei and occupied by fine fibrils.  $\times 700$ .

FIG. 28, *a* and *b*. Stages in the formation of the branchial orifice. In *a* the much thickened invaginated ectoderm, *e.br.o.*, is seen nearly touching the branchial wall. The fusion of the two walls is shown in *b*.  $\times 700$ .

FIG. 29, *a* and *b*. These sections illustrate the development of the branchial stigmata. In *a* the thickened branchial wall, *br.w.*, is slightly evaginated, and is in contact with the thickened patch of cells in the visceral wall of the peribranchial sac, *pb.w.* The lower portion of *b* shows the fusion between the two walls, and the upper portion, a stage after the formation of the opening.  $\times 700$ .

FIG. 30. Section through the wall of the stomach, *st.w.*, and a portion of the duct of the "organe réfringent," *o.r.r.*, showing clearly the connection of the latter with the digestive tract.  $\times 700$ .

FIG. 31. Transverse section of the terminal portion of the intestine, *int.*, showing the surrounding ducts and ampullae of the "organe réfringent," with their deeply stained nuclei, *amp.*  $\times 700$ .

FIG. 32, *a, b*, and *c*. Sections illustrating the early development of the sexual organs. In *a* an early stage is shown when the rudiment, *g.r.*, is merely a loose mass of cells surrounding a slight cavity, *c.g.r.* A couple of the elongated cells which will help to form the genital cord are seen at *g.c.* In *b* the cavity of the rudiment is much enlarged, and the genital cord is prominent, and attached to the sphere; in both these figures the genital rudiment is seen to lie close to the wall of the digestive tract. In *c* an older stage is shown, in which the cavity of the sphere has become divided into two, *c* and *c'*; the genital cord, although only represented in part, now extends nearly to the posterior wall of the cloaca, and lies close to and parallel with the intestine.  $\times 700$ .







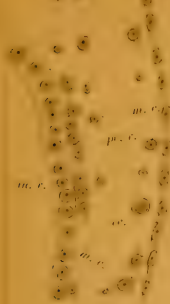


Fig. 251.

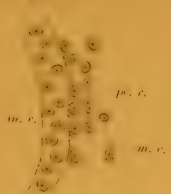


Fig. 5 c.

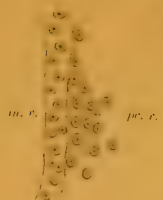


Fig. 26 a.



Fig. 26 b.

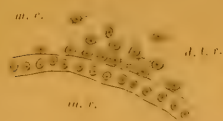


Fig. 2 a.

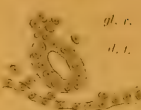

$$T_{ij} = \frac{1}{2} \rho v_i v_j$$


Fig. 20 a.

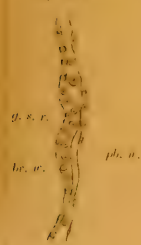


Fig. 5 d.



Fig. 25 c.



Fig. 26 c.



Fig. 9: d.

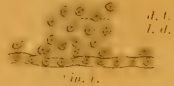


Fig. 26 c.



Fig. 31.



Fig. 2 c.

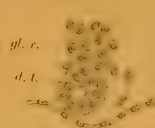


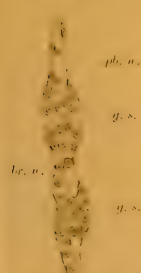
Fig. 27 d



Fig. 26 c.



*Fig. 29 b.*



*Fig. 28 a.*

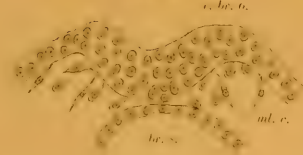


Fig. 30.



Fig. 32 a.

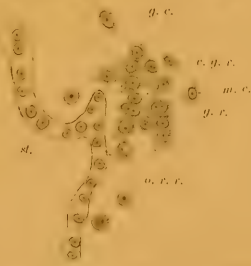
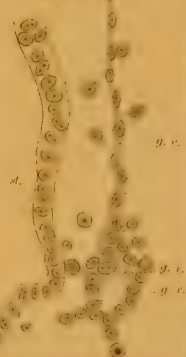


Fig. 32 b.





## ON THE MORPHOLOGY OF CERTAIN OF THE BONES OF THE CHEEK AND SNOUT OF AMIA CALVA.

EDWARD PHELPS ALLIS, JR.

THE bones it is my purpose here to consider are, in Sagemehl's nomenclature (Nos. 35, 36), the preoperculum, the postfrontal, the infraorbital and antorbital chains of bones, the prefrontal, the nasal, the vomer, and the maxillary bones. The infraorbital bones are the two postorbitals, the two suborbitals, and the lachrymal. The antorbital bones are the antorbital and the median ethmoid. The maxillary bones are the maxillary, the premaxillary, the septomaxillary, and the supramaxillary. The supramaxillary of this nomenclature is the jugal of both Bridge's descriptions and my own (Nos. 7, 3). The prefrontal is the lateral ethmoid or ectethmoid of most English writers, and is the preorbital ossification of all my figures, though often, in my descriptions, called by me, as by Sagemehl, the prefrontal.

Of these several bones, the preorbital ossification and septomaxillary are cartilage bones, or so-called primary ossifications; that is, they are developed in direct relation to, and replace parts of the chondrocranium. Whether they are, in *Amia*, of endochondral or perichondral origin I have not yet attempted to investigate.

The remaining bones are all dermal bones, or so-called secondary ossifications, but they belong, by origin, to two somewhat different categories.

The maxillary, premaxillary, and vomer all bear teeth, and hence belong, beyond question, to those bones that are developed in direct or indirect connection with the teeth of the animal. The supramaxillary bears no teeth, but its homologue in teleosts is considered by Sagemehl as phylogenetically derived from teeth-bearing plates similar to and continuous with those that, according to him, fuse to form the maxillary (No. 36, p. 101).

The preoperculum, postfrontal and nasal, and the infraorbital and antorbital bones are all traversed by canals of the lateral-line system, and are all developed in definite topographical relation to the sensory organs found in those canals (No. 1, p. 496).

The teeth-bearing bones of vertebrates are all said by Hertwig to arise by the direct fusion of the cement plates that form the sockets of the teeth. Exactly the same origin is also assigned by him to those bones of the mouth cavity that do not, in certain vertebrates, either in embryos or in the adult, bear teeth, and also to all the dermal bones, or dermal components of the bones, of the outer surface of the skull; the process in these latter cases being said to be simply abbreviated (No. 18, p. 568). This theory of Hertwig, based upon his own investigations on selachians and urodeles, is said by Klaatsch to be fully confirmed, in so far as it relates to the bones of the mouth cavity, by the development of the bones in teleosts (No. 22, p. 210). The bones in these latter fishes are, however, said by him to arise, not only from the fusion of the cement plates that form the sockets of the teeth, but also by the direct absorption or incorporation of certain of the teeth or teeth-anlagen themselves. Röse (No. 33) finds further confirmation of the theory, as applied to the bones of the mouth cavity, in the development of the teeth of the crocodile, and further adds that Hertwig's theory, in so far as it relates to these particular bones, should now be accepted as an established scientific fact. With these conclusions Walther's observations, made several years before those of the last two writers, are not fully in accord; for, although a pupil and follower of Hertwig, he says (No. 46) that, in teleosts, the teeth and their cement plates, and the bones that underlie and ultimately bear them, have a certain independence in their origin and development not found, or not noticed, by Hertwig in the animals investigated by him. Carlsson has lately more than confirmed this statement of Walther's, for she says (No. 9, pp. 237, 240) that the teeth and the teeth-bearing bones of teleosts have, genetically, a complete and absolute independence; adding, furthermore, that this same independence of origin has been



established for them, by Leche, Röse, and others, in mammals also. It is thus evident that while, if Hertwig, Klaatsch, and Röse are right, the teeth-bearing bones of animals can contain, genetically, but one component, they may, or must, contain two such components if Walther and Carlsson are right. Klaatsch's observations on the development of the canal bones of teleosts seem to indirectly confirm the presence of these two components, notwithstanding the fact that he himself makes such a positive statement to the contrary.

The canal bones of fishes, that is, all those bones that are traversed by the canals of the lateral-line system, are said by Klaatsch (No. 22, pp. 203, 211) to be developed directly from scleroblastic cells proliferated in direct connection with the sense organs contained in the canal or canals by which the bone is traversed. This origin of the bones definitely excludes, according to him, all possibility of that part of these bones that immediately surrounds the canal being formed, in any part even, by the fusion of dermal teeth or teeth-bearing plates. Where the bone traversed by the canal is large, and extends for some distance on one or both sides of the line of the canal to which it is related, such distant parts either arise from scleroblastic cells that have migrated thither from the related sense organs as proliferating centers; or the bone adjoining the canal undergoes an enormous development and displaces — “auf Kosten” is the expression used — other neighboring bones that may perhaps have arisen in connection with dermal teeth. The possibility of other bones of somewhat different origin preëxisting in these regions is thus admitted.

With these conclusions of Klaatsch, and also with the statements of facts on which they are based, the observations and conclusions of certain other writers are not entirely in accord. Walther (No. 46, pp. 66, 78) considers the frontal and nasal bones of teleosts, both of which are traversed by lateral canals, as of connective-tissue origin, but phylogenetically derived from tooth-bearing plates; and he says of the frontal of *Esox* (No. 46, p. 72) that that part of the bone of the adult that encloses the lateral canal to which it is related, begins its development as two little processes rising perpendicularly from the surface

of an already formed plate of connective-tissue origin. Schmid-Monnard (No. 38, pp. 109, 116) says, that, while that part of the squamosal of *Salmo* and *Esox* that immediately surrounds the lateral canal by which the bone is traversed is of osteoblastic origin, other parts of the bone are of purely membranous origin, arise independently of the part enclosing the canal, and then fuse with it. In *Esox*, that part of the squamosal that forms the articular facet for the hyomandibular is even said by him to acquire, secondarily, a cartilaginous character, and hence to represent a third, histologically different if not independent, component of the bone. McMurrich (No. 23, p. 297), before Schmid-Monnard, described the first two of these three components as primarily separate and independent parts in several of the canal bones of larvae of *Amiurus*, and I find the same conditions in larvae of *Amia*. The frontal, for example, in 20 mm. larvae of *Amia*, consists of two wholly separate parts, a semicylindrical gutter lying immediately beneath the supraorbital canal, and a flat, plate-like portion lying mesial to the canal, directly upon the cartilage of the chondrocranium. Still further evidence of the existence and primary independence of two components in these bones seems to be found in Sagemehl's statement (No. 36, p. 38) that in certain teleosts the lateral canals are found enclosed in bony tubes, which lie wholly superficial to, and independent of the bones that underlie them. This condition, which Sagemehl considers as secondary, is thus, probably, simply a persistence of the primary, independent condition of the two components of the bones. Röse (No. 34, p. 661) considers the observations on which Klaatsch bases his conclusions as wholly erroneous; and Gaupp (No. 17, p. 80) thinks that they need confirmation.

In *Amia*, and hence, doubtless, in all fishes, those particular parts of each canal bone that lie immediately beneath the sense organs to which they are related begin to develop while those sense organs are still exposed on the outer surface of the head of the fish. It is, therefore, certain that the development of the bone does not depend, genetically, in any way upon the ultimate formation of a canal. Bones that are strictly homologous may, accordingly, be found in certain fishes related to sense

organs enclosed in sections of canals, and in other fishes related to corresponding lines of organs which remain permanently exposed on the outer surface of the head. Such exposed organs are found in *Amia* in the pit organs of my descriptions (No. 1, p. 502), and the parietal of *Amia*, which lies immediately beneath certain of these organs, is apparently a bone developed in relation to them; for the parietal of *Amia*, although it encloses a terminal portion of the terminal dendritic system of the supraorbital canal, lodges no enclosed sense organ of the line. The supra-angular of *Amia*, which is similarly related to one of the dendritic systems of the preoperculomandibular line of sense organs, may possibly be similarly related, in its development, either to the vertical cheek line, or to the mandibular line of pit organs, notwithstanding the fact that the bone does not, in the adult fish, lie directly internal to either of those lines of organs. In larvae of *Amia* the two lines of organs form a continuous line extending from organ 8 mandibular to organ 12 infraorbital (No. 1, p. 534).

Klaatsch, in the figures illustrating his descriptions, shows no terminal buds, canal organs alone, among sense organs, being shown as centers from which scleroblastic cells are proliferated. In his descriptions the terms "*Hautsinnesorgane*" and "*Sinnesknospe*" do not definitely indicate whether he refers to canal organs alone, or to terminal buds also. He, however, definitely says (No. 22, p. 220) that what is true for the canal bones is true also "*zum grossen Theil*" for the opercular bones; and the operculum of *Amia*, and of all teleosts, also, so far as I can find, has no direct relation whatever to any part of the lateral-line system. The scleroblastic cells, to which, according to Klaatsch, this bone owes its origin, must, therefore, either arise from the terminal buds that lie in great numbers directly superficial to it, or they must have migrated beneath the bone from the canal organs of that canal of the lateral-line system that traverses the wholly separate and independent preoperculum. The latter supposition seems improbable, and Klaatsch's special reference (No. 22, p. 201) to the development of the bone seems to definitely exclude it. One is, therefore, led to conclude that if Klaatsch's observations and conclusions are

correct certain of the dermal bones of fishes must be developed in connection with terminal buds, just as others are developed in connection with canal organs and pit organs. While this might perhaps be assumed, without discussion, from the similarity alone of the organs, it is not to be overlooked that, so far as is at present known, the two sets of organs are innervated by entirely separate and distinct nerves (No. 3); and that, accordingly, if they both are centers of scleroblastic proliferation, the bones, or parts of bones, arising in connection with each may have a certain independence. Moreover, if terminal buds are such centers of proliferation, certain of the teeth-bearing bones, as well as the canal bones, must arise, in part, in connection with them; for terminal buds are especially numerous immediately superficial to all those bones that take part in the formation of the margins of the mouth.

Whatever the definite origin of the two kinds of bones here under consideration may be, it is certain that both the canal bones and the teeth-bearing bones of fishes are remarkably constant elements of the skull of vertebrates; that they may be found even when the teeth or the sense organs to which they are supposed to owe their origin do not develop, or at least do not persist; and that the individual bones of each class possess inherently, in early phylogenetic stages, not only the possibility of fusion with each other, but also of fusion with bones of the other class. This latter proposition is proved conclusively by the dentary of *Amia* and other fishes, by the so-called maxillary and premaxillary bones of *Polypterus* (No. 44), and by the so-called maxillary chain of bones of *Lepidosteus* (No. 28, p. 478), all of which bones both bear teeth and lodge important parts of the lateral canals. Furthermore, it is highly probable, though certainly not yet established, that a bone or part of a bone developed in any particular fish in relation to a particular part of the lateral-line system is always the homologue of the bone, or of the part of a bone, developed in relation to the same part of the lateral-line system in any other fish or animal.

It may furthermore be stated that the canal bones and teeth-bearing bones of vertebrates arise, according to Gegenbaur, phylogenetically before the so-called primary ossifications;



that, in Sagemehl's opinion (No. 36, p. 98), they are all developed in relation to certain definite underlying parts of the cartilaginous cranium, with which, if they do not entirely displace and replace them, they may acquire so-called primary relations; that certain of them may acquire relations to other cartilaginous parts than those to which they were primarily related (No. 17, p. 82); and that, in general, they acquire, because of their great persistence, independence and individuality, and their relations to each other, an ever increasing importance in the formation of the skull (No. 17, pp. 83, 84).

The several single bones with which I am here concerned can now be considered. They have all been already described in *Amia* by Bridge (No. 7), and part of them by Sagemehl also (No. 35). The comparisons I shall attempt to make of the conditions found in *Amia* with those found in other animals are limited almost entirely to other fishes, to certain fossil reptiles, and to man. That the apparent homologies found between certain of the bones, or combinations of bones, of *Amia* and other fishes and those of man cannot be considered as real until they have been traced through other classes is evident. They are, however, in many cases sufficiently striking to warrant their being presented.

The nasal (*NA*, Pl. XXXIII, Fig. 1) of *Amia* is a slightly convex and irregularly oval bone which covers almost completely the relatively large and flat olfactory pit. Laterally it adjoins the antorbital bone; antero-mesially it overlaps the postero-lateral edge of the ethmoid; and posteriorly it overlaps two thin processes of the frontal, which project forward, the one from the lateral edge of the bone, and the other from its mesial edge. Both of these processes arise from the deeper layers of the frontal, lie below the level of the outer surface of the rest of the bone, and have not, on their dorsal surfaces, the sculptured markings peculiar to all the dermal bones of the outer surface of the skull of *Amia*. The lateral process rests directly upon the dorsal surface of the preorbital ossification, and reaches or passes slightly beyond the dorso-posterior end of the antorbital bone, which bone will be later shown to be the probable homologue of the nasal process of the superior maxillary bone of man. As the



preorbital ossifications are generally considered as, and probably are, the homologues of the lateral masses of the ethmoid bone of man, the two processes of the two frontals of *Amia* correspond, one or both of them, to the nasal spine or nasal process of the single frontal bone of the adult man.

Between the nasal of *Amia* and the body of the frontal there is everywhere a relatively wide strip of tough dermal tissue. Medianly, the nasal adjoins its fellow of the opposite side of the head, the two bones resting upon connective tissue that covers the narrow, dorsal edge of the median, cartilaginous wall that separates the nasal fossae. To all the bones that adjoin it, excepting only the frontal, the nasal is bound by tough fibrous tissue, and it nowhere rests directly upon the cartilage of the chondrocranium. It is traversed by the anterior part of the supraorbital lateral canal.

In *Polypterus* (No. 44, p. 173), instead of a single nasal bone on each side of the head, as in *Amia*, there are two such bones, both of which are said to be traversed by the supraorbital lateral canal. In front of the anterior, or accessory-nasal bone the supraorbital canals of opposite sides of the head are said to form a transverse commissure through the ethmoid. Connected with the anterior nasal there is a small bone said by Traquair to be "connected with the termination of the main lateral mucous canal of each side." It is called by him the *os terminale*. By Erdl (No. 13, p. 242) it is considered as the true nasal bone, the large posterior nasal bone of Traquair being considered as the *pars nasalis ossis frontis*. From the somewhat indefinite descriptions of it, it seems to have no separate homologue in *Amia*.

In *Lepidosteus*, as in *Polypterus*, there are two nasal bones on each side of the head, the larger and posterior of the two being called by Parker the ethmo-nasal (No. 28, p. 477). The latter bone is said by van Wijhe to be traversed by the supraorbital lateral canal (No. 48, p. 273). Whether the smaller, anterior bone is or is not so traversed is not stated by van Wijhe, and is uncertain both in his descriptions and in Parker's figures. A sketch that I have, made several years ago, of a young *Lepidosteus* shows a canal in the bone in question, but leaves it wholly uncertain to which of the main canals it belongs.

In *Amiurus catus*, the adnasal bone of McMurrich is said by him (No. 24, p. 278) to contain a part of the infraorbital lateral canal. It accordingly belongs, if McMurrich is correct, to the infraorbital, or antorbital, chain of bones, and not, with the nasal, to the supraorbital chain.

The ethmoid (*ETH*) of *Amia* is a median V-shaped bone that lies between and in front of the nasals. It rests, as the median edges of the nasals do, upon connective tissues that cover the dorsal surface of the median, rostral process of the chondrocranium, and, like the nasals, it does not touch the cartilage at any place. The anterior end of the rostral process of the chondrocranium reaches forward to the point where the two arms of the bone diverge, but is not exposed between them on the dorsal surface of the skull. The anterior end of each arm of the bone rests upon, and is bound by dermal and fibrous tissue to, the dorsal surface of the corresponding premaxillary, immediately above and behind the teeth of the latter bone. Laterally, the anterior end of each arm of the ethmoid touches, and is bound by dermal tissue to, the anterior end of the corresponding antorbital bone. The bone is traversed by the anterior cross-commissure of the infraorbital lateral canal, and, being developed in connection with that canal, is unquestionably formed by the fusion of two lateral components, one on each side of the head.

In *Polypterus*, according to Pollard (No. 30, p. 400), there is, as in *Amia*, a median, dermal ethmoid, traversed, as in *Amia*, by the anterior cross-commissure of the infraorbital canal. According to Traquair (No. 44, p. 171), this bone is of primary origin, but has a flat, narrow, posterior process which ossifies "in the membrane superficial to the cartilage." According to Pollard, the bone lies wholly on the dorsal surface of the skull. According to Traquair, it forms part of the ventral as well as part of the dorsal surface of the skull. According to Erdl (No. 13, p. 242), it is the homologue of the vomer bone of other fishes, and is not in any sense an ethmoid bone.

In teleosts, the so-called ethmoid is said to be sometimes a purely dermal bone (No. 26); sometimes partly of dermal and partly of cartilaginous origin; sometimes entirely of cartilaginous

origin (Nos. 36, 37); and sometimes wholly wanting (No. 13, p. 242). In no teleost is it known to be traversed by an anterior cross-commissure of the lateral-line canals, that commissure, as a canal, not existing in teleosts, so far as I can find described. There is, however, in some teleosts, an anterior cross-commissure of surface sense organs, corresponding exactly, in its relations to the canals of the lateral line system, to the commissural canal of *Amia* (No. 1, p. 471); and in those fishes in which I definitely know this commissure to be found it is, with one apparent exception, *Amiurus*, associated with a purely dermal ethmoid. Sketches that I have, made several years ago, show it in *Salvelinus namaycush* and in *Salmo fontinalis*; and in *Salmo salar*, a fish so closely related to these two that it must be found in it also, there is, underlying the region where the line should be found, a purely dermal bone, the supra-ethmoid of Parker's descriptions (No. 26). A similar bone is found in *Micropterus salmoides* (No. 41, p. 60); and in *Micropterus dolomieu* my sketches show a cross-commissure of surface organs. In *Amiurus catus* and *Silurus glanis* my sketches show, on each side of the head, surface organs which represent the proximal parts of a cross-commissure, if not the entire commissure; and in *Amiurus*, McMurrich says of the mesethmoid that it "is one of the two bones in which the ossification of the cartilage is not completed in the adult, the inner surface of the bone being lined with it" (No. 24, p. 276). In *Clarius*, a fish somewhat related to *Amiurus*, there is, according to Bridge (No. 7, p. 609), a median, T-shaped, dermal ethmoid.

In *Salmo salar*, the supra-ethmoid of Parker overlaps externally the anterior ends of the frontals (No. 26, Pl. VII, Fig. 1). In *Amia* the ethmoid would naturally have a similar relation to the thin, anterior processes of the frontals if it were produced backward between the nasals so as to reach the former bones. In larvae of *Salmo salar* (No. 45, Fig. 37) the ethmoid seems to lie, as it does in the adult *Amia*, considerably in front of the frontals.

In *Esox lucius* I find bones 2 of Huxley's descriptions (No. 21, p. 134) lying immediately beneath lines of surface sense organs, which are somewhat longitudinal in direction and do not form a



cross-commissure across the top of the head. The mesial part of each of these bones lies directly upon the dorsal surface of a long, thin, anterior end, or process, of the frontal, which process lies directly upon the cartilage of the snout. Lateral to this process, bone 2 rests directly upon the cartilage of the dorsal surface of the snout and upon the dorsal surface of bone 3, which bone both Bridge and Sagemehl consider as the homologue of the septomaxillary of *Amia*. The posterior part of bone 2 is covered externally by the anterior half or two-thirds of the nasal, but the bone itself forms no part of the roof of the nasal capsule, its lateral edge turning downward along the shelving, lateral surface of the cartilage of the snout. Its antero-lateral extremity articulates with the premaxillary, and is bound to that bone, and to the palatine bone behind it, by strong fibrous or ligamentous tissue. In one large specimen the septomaxillary lay slightly below the level of the dorsal surface of the rostrum, and there was a corresponding raised surface on the ventral surface of bone 2, which fitted into the depression and onto the septomaxillary. The adjoining surfaces of these parts of the two bones were rough, projecting points on one fitting into depressions on the other, thus strongly suggesting the beginning of a process of ankylosis.

Bone 2 of *Esox* thus has the same general relations to the adjoining bones and parts of the skull that each half of the single ethmoids of *Amia* and *Salmo* have, the relation to the septomaxillary in *Amia* alone excepted. I, therefore, consider the two bones together of *Esox* as the probable homologue of the single bones of *Amia* and *Salmo*. The only other supposition possible, apart from that made by Huxley, is that each bone in *Esox* is the homologue of the posterior process of the premaxillary of *Amia*; a supposition which, from the different relations of the two bones to the frontals in their respective fishes, seems much less probable. That there should be two ethmoid bones in *Esox* instead of one, as in *Amia*, seems to be sufficiently accounted for by the fact that the lines of sense organs to which the bone seems to be related, in *Esox*, are longitudinal in their general arrangement and not transverse. If the two bones of *Esox* are parts of a dermal ethmoid bone of that fish, instead of parts of the pre-

maxillaries, or of the nasals, as Huxley was inclined to think, their relations to the septomaxillaries must be of secondary origin, due to the great lengthening of the snout.

In the Cyprinidae and Characinidae the ethmoid is a median bone. In the Cyprinidae it is said by Sagemehl (No. 37, p. 497) to lie ventral to the anterior ends of the frontals. In the Characinidae (No. 36, p. 30) one is led to suppose that it has the same relative position to those bones, although this is not definitely said by Sagemehl to be so. In none of these fishes is the bone traversed by any portion of the lateral canals, and no intimation is given by Sagemehl of any lines of surface sense organs in relation to it. Such lines may, nevertheless, exist, for Sagemehl in his investigations took no account of them. In both the Cyprinidae and the Characinidae, the ethmoid, according to Sagemehl, is either partly of primary origin or has acquired more or less intimate primary relations to the chondrocranium.

In Scomber, Dr. Dewitz finds a median ethmoid, and it has the same relation to the frontal bones of the fish that the ethmoid has in the Cyprinidae and Characinidae; that is, it lies wholly ventral to them. The bone, in Scomber, is certainly in largest part, if not entirely, of primary origin, and, as might have been expected, Dr. Dewitz has been wholly unable to find any trace whatever of pit organs in relation to it.

These several facts all seem to indicate that the so-called ethmoids of *Amia* and *Salmo*, and bones 2 of *Esox*, which I consider as the ethmoid of that fish, are not the homologues of any part of the ethmoid of Scomber. Other facts, which will be given below in treating of the premaxillary bone, indicate that they are also not the homologues of any part of the ethmoids of the Characinidae and Cyprinidae, but this is much less certain than in the case of Scomber. In the Cyprinidae, in particular, the ethmoid has, according to Sagemehl, two such well-marked components that the superficial one, notwithstanding its position internal to the frontal, may be, as Sagemehl states, the homologue of the bone of *Amia*.

The antorbital bone (*ANT*) of *Amia* is a long bone, the anterior end and anterior part of the lateral edge of which rest upon the dorsal surface of the premaxillary, immediately above and



behind the teeth of that bone. Its anterior end adjoins mesially, and is bound by tissue to, the anterior end of the ethmoid. In its posterior part it adjoins the nasal mesially and the lachrymal postero-laterally. Its posterior end is directed toward, but does not reach, the antero-lateral corner of the body of the frontal. Postero-lateral to this end of the bone, between it and the lachrymal, is the posterior nasal aperture; the canal leading from that aperture into the nasal sac running mesially and forward posterior to and then mesial to the hind end of the antorbital. Between the anterior end of the bone and the anterior ends of the ethmoid and nasal is the anterior nasal aperture. The lateral edge of the bone is bound by fibrous or ligamentous tissue to the anterior end of the maxillary. The bone is traversed by the proximal portion of the anterior cross-commissure of the infraorbital lateral canal, and encloses, perhaps, also, the extreme anterior end of the latter canal itself.

The antorbital bone, as a separate bone, cannot be positively recognized in any of the descriptions that I have of other fishes. According to Bridge (No. 7, p. 609), it is described by Huxley in *Clarias* as the preorbital bone. This bone of *Clarias* is shown by Pollard in a figure intended simply to show the course of the lateral-line canals of that fish (No. 31, Pl. XXXV, Fig. 1), and is called by him the antorbital bone. He, however, says in the text that there is another, small, rudimentary, dermal bone lying in front of it, between it and the antero-lateral end of the ethmoid. As the lachrymal of his figure lies partly below the eye, and but one suborbital bone is given, his lachrymal may be the homologue of the first suborbital bone of *Amia*; his antorbital then being the lachrymal of *Amia*, and the rudimentary bone the antorbital.

Similarly, in the Cyprinidae the several references made by Sagemehl (No. 37, pp. 566, 567, 587) to a preorbital bone are insufficient to warrant its identification as the antorbital of *Amia* rather than the lachrymal. The bone is said to be the most anterior bone of the "Orbitalbogen," thus occupying exactly the place of the lachrymal in *Amia*; and the use of the term preorbital instead of antorbital indicates in itself that the bone could not have been considered by Sagemehl himself as the

homologue of a bone to which he himself had given a different name (antorbital) in *Amia*.

In *Lepidosteus*, the preorbital bone of Parker (No. 28) seems also to be a lachrymal rather than an antorbital, the latter bone being apparently represented in some part of the long chain of teeth-bearing, so-called, maxillary bones.

In *Amiurus*, the adnasal bone of McMurrich (No. 24, p. 278) has the position of an antorbital bone, and Collinge says (No. 12, p. 280) that it is so called by some authors.

In certain other fishes, where neither an antorbital nor a preorbital bone is described, the antorbital may, perhaps, have fused with the lachrymal to form the long anterior part of the bone so named; as, for examples, in the haddock (No. 8, Fig. 4) and in *Esox*. In *Polypterus* it seems to have fused with the premaxillary instead of with the lachrymal, as a comparison of my figure of the skull of *Amia* (No. 3, Fig. 1) with Traquair's figure of *Polypterus* (No. 44, Fig. 7) will plainly show. Aside from the striking topographical resemblance of the wholly separate bone of *Amia* to the postero-lateral process of the premaxillary of *Polypterus*, the fact that the process of the latter bone in *Polypterus*, as well as a part of the body of the bone itself, is traversed by the infraorbital lateral canal is of the greatest importance. That this canal could traverse a bone strictly homologous with the premaxillary of *Amia* and other fishes is, from the origin ascribed to the bones, if it be correct, wholly impossible.

The postfrontal and infraorbital bones together of *Amia* are six in number; the postfrontal (*PSF*), the lachrymal (*LA*), the two suborbitals (*SOR*), and the two postorbitals (*POR*). The same number of bones are found in *Salmo* (No. 26), in *Amiurus* (No. 24, p. 277), and in the haddock (No. 8).

In *Amia* the lachrymal and first suborbital overlap externally the anterior half of the maxillary; the supramaxillary overlaps externally the ventral edges of the second suborbital and first postorbital; and the bones of the two series are connected through the greater part of their length by fibrous or ligamentous tissues. In the haddock, where there seems to be no supramaxillary, the maxillary, judging from Brooks' figure, must, when

the mouth is closed, lie entirely internal to the lachrymal and first suborbital. In *Polypterus* the so-called maxillary occupies a position corresponding exactly to that of the maxillary, supra-maxillary, and suborbital bones together of *Amia* when the mouth is closed, and it is traversed, as the premaxillary in the same fish is, by the infraorbital lateral canal. That this canal could traverse a bone homologous with the maxillary of *Amia* is even more improbable than in the case of the premaxillary. The so-called maxillary of *Polypterus* must, accordingly, either be formed by the fusion of the maxillary bones of *Amia* with certain of the infraorbital bones of the same fish, or the maxillary bones must either have disappeared in *Polypterus*, or not yet have developed as separate bones, and the infraorbital bones must have independently acquired teeth, as the antorbital canal bones of *Lepidosteus* seem to have done. The former supposition seems much the more probable, and I assume it to be true. As the so-called anterior suborbital bone of Traquair's descriptions of *Polypterus* occupies exactly the position of the lachrymal of *Amia*, and is traversed by the infraorbital lateral canal after that canal has left the so-called maxillary, and before it enters the premaxillary (No. 44, p. 181), the fusion of the canal bones with the teeth-bearing ones must begin with a homologue of one of the suborbital bones of *Amia*.

Regarding the postfrontal I have nothing to add to what I have already said about it in an earlier work (No. 1, p. 478). So far as my experience goes, it never in any fish fuses with the underlying postorbital ossification. On the contrary, in *Scomber*, where the muscles of the cheek acquire an origin in part from the lateral edge of the dorsal surface of the skull, as they do in the *Characinidae* and *Cyprinidae* (No. 36, p. 61; No. 37, p. 501), the postfrontal, definitely identified by the canal that traverses it, is found on the outer surface of the muscles and not beneath them.

No separate description of the maxillary and supramaxillary is necessary.

The preoperculum (*POP*) is traversed its full length by a lateral canal and is developed in connection with, or in relation to, the sense organs of that canal. According to Sagemehl



(No. 36, p. 96) it is formed by the fusion of several bones, some of which are still found separate and independent in certain fishes.

The canal that traverses the bone or bones was called by me in my earlier work (No. 1) the opercular part of the operculo-mandibular lateral canal. For the latter canal I now propose the name preoperculo-mandibular canal, and for its two parts the names preopercular canal and mandibular canal. The reason is evident, the canal always traversing or lying in the preoperculum, and not the operculum. That a double name for the whole canal, or, if a single name is used, two separate names for the two parts of the canal, is necessary, is evident from the fact that the canal develops as two wholly separate and independent parts, which may or may not later unite to form a single canal (No. 1, p. 529).

For this entire canal, Ewart (No. 14), who takes exception to the name adopted by me in my earlier work, has proposed the name hyomandibular canal, this name being based on the name of the nerve that innervates the organs of the line. In sharks other than *Laemargus* (No. 14, Pl. II, Fig. 2) he, however, gives the name hyomandibular more particularly to what is apparently the homologue of the preopercular part only of the canal in *Amia*; calling what is apparently the homologue of the remaining, lower and distal part of the canal of *Amia* the mandibular canal, as I did in *Amia*. Collinge (Nos. 11, 12) adopts the name hyomandibular for the entire canal, but uses that name interchangeably with operculo-mandibular in the fishes described by him. Platt (No. 29, p. 494) adopts the name hyomandibular, as proposed by Ewart, but apparently applies it in *Necturus*, as applied by Ewart in sharks in general, to the preopercular part only of the canal of *Amia*; stating, moreover, that the line of sense organs so designated in *Necturus* is the homologue of the operculo-mandibular line of my descriptions of *Amia*, that is, of the entire line. The only other supposition possible, and perhaps the correct one, is that the so-called mandibular line of *Necturus* is the homologue of the cheek and mandibular lines of pit organs of *Amia*.

The hyomandibular line of Platt, in *Necturus*, is said by her to be innervated by the hyomandibularis facialis, the ven-

tral continuation of which nerve is said to be the mandibularis internus facialis. This latter nerve is said to innervate, among other organs, an inner row of mandibular sense organs lying along the margin of the lower lip. Another and more important row of mandibular organs is said to be innervated by the mandibularis facialis, this same nerve being later referred to as the external mandibular nerve (No. 29, pp. 531, 534). In *Amia*, both the preopercular and mandibular parts of the main line, and also the associated lines of pit organs, are all innervated by the mandibularis externus facialis alone, the mandibularis internus facialis innervating no surface sense organs whatever, unless it be certain of the terminal buds (No. 3, p. 634). Confusion, therefore, at once arises, and to attempt to give definitely to these and other lines of sense organs, in fishes and other animals, the names of the nerves that innervate them before the homologies of the nerves and organs are well established, seems to me most unwise. Moreover, names based simply on the names of the nerves that innervate the different canals are not, in fishes, sufficiently definite for detailed descriptions, and recourse must still be had to other terms. I, therefore, adhere for the present as closely as possible to the purely topographical method of naming adopted in my earlier work, using the innervation of the organs of the different parts of the system for comparative rather than descriptive purposes.

Connected with the sense organs of the preopercular canal there is, in *Amia*, a horizontal line of pit organs, above referred to, which extends across the cheek from among those surface openings of the dendritic systems of the preoperculo-mandibular line that form groups 14 and 15 of my descriptions to those that form group 12 infraorbital (No. 1, p. 506). In four-day-old specimens this line is almost a direct continuation of a dorsal part of the preopercular canal line, that part of the canal line being distinctly separate from the ventral part (No. 1, p. 534, Fig. 2). In part of its course this line of organs lies directly superficial to the lower postorbital bone. In *Esox* the corresponding line has moved bodily forward, ventral to the line of the suborbital lateral canal, and lies superficial to the lachry-



mal, or to that bone and the suborbital bones. In Chimaera, what seems to be this same line of organs is said by Cole (No. 10) to be a ventral division of the infraorbital part of the main lateral line, and to be innervated, as the dorsal part of the line is, by branches of the buccalis facialis; a special branch of the main nerve being differentiated for the innervation of the organs of the ventral line and those of the associated group of ampullae. In Polyodon, what seems to be the corresponding line is enclosed in a canal (No. 11, p. 512). As all of the sense organs of the lateral canals, both in Polyodon and in Chimaera, are said to be partly enclosed in bone (No. 11, and No. 1, p. 496), it is to be presumed that the particular organs here under consideration are so enclosed. Bone thus being, presumably, formed in these two fishes in connection with this line of organs, is it not highly probable, wholly apart from Klaatsch's opinion of the scleroblastic character of the organs, that bone should be also formed in connection with it in certain other fishes, or other animals? And that the bone once so formed might still persist even after the disappearance of the organs to which it owed, primarily, its origin? The conditions found in Polypterus and in the Stegocephali and other reptiles seem to indicate that such is the case.

In *Trematosaurus braunii*, the preopercular lateral canal begins, according to Baur (No. 5), in the prosquamosal, runs downward, and downward and forward, through that bone, and then enters the jugal, where it joins and becomes part of the suborbital part of the main infraorbital canal of the animal. The united canals then run forward through the jugal into the maxillary, and end near the anterior end of the latter bone. No mandibular canal is given by Baur, or in any way indicated, either in the descriptions or in the figure. Because of the canal that traverses it, the prosquamosal is said by Baur to be the homologue of the preoperculum of fishes.

A bony connection is thus found established in *Trematosaurus* between the preoperculum and the suborbital chain of bones; and it is traversed by a lateral canal, which connects the preopercular and suborbital canals. A similar canal is said by Pollard (No. 31, pp. 546, 548) to be found both in Coc-

costeus and Chlamydoselachus, the canal in the former animal traversing the so-called supratemporal bone, which Pollard considers the homologue of the preoperculum. In Polypterus, also, a bony connection is found at this same place, wholly unassociated with a lateral canal, according to Traquair's descriptions, but, according to Pollard (No. 31, p. 548), associated with rudiments of such a canal. The prosquamosal of Trematosaurus and the supratemporal of Coccoosteus are certainly the homologues of the supratemporal of Capitosaurus, which bone also forms a bony connection between the two lines of bones here under consideration. This bone of Capitosaurus, which is not said to be traversed by a lateral canal, is considered by Gaupp (No. 17, p. 105, Pl. VII, Fig. 11) as the homologue of the paraquadratum of his descriptions of other vertebrates.

In Trematosaurus, the preopercular canal, after leaving the prosquamosal, enters, as already stated, the jugal, which bone extends forward under and beyond the eye, but lies wholly posterior to the maxillary bone. The jugal of Trematosaurus is thus the apparent homologue of that part of the compound, so-called maxillary bone of Polypterus that lies either posterior to, or postero-superior to, the maxillary element of that bone, fused, perhaps, with one or with both of the bones designated by Traquair as bones  $j'$  and  $j''$ . What the homologue, in Polypterus or in Amia, of the quadrato-jugal of Trematosaurus may be is not evident. In Polypterus it may be represented by bone  $j''$ . It is not traversed in Trematosaurus by the preopercular canal of that animal, and hence cannot be one of the bones developed in connection with that line of organs. As it becomes intercalated, in certain reptiles, between the prosquamosal and jugal (No. 6, Fig. 1), it may possibly represent that part of the preoperculum of Amia that lies distal to the horizontal cheek lines of pit organs, or be a bone that is developed in connection with the vertical and mandibular cheek lines of organs, and either not found as a separate bone in the skull of Amia or found as the supra-angular. It is to be noted that the quadrato-jugal in the Stegocephali may take part in the formation of the mandibular articulation, either as "Ansatz an das Quadratbein

oder an den Unterkiefer" (No. 16, p. 46), and that in *Amia* the lower end of the preoperculum supports intimately the lower end of the symplectic, where that bone articulates with the mandible. If the lower portion of the dermal preoperculum of *Amia* should here fuse with the underlying cartilage bone, or simply replace it, both of which processes are said to be of frequent occurrence in other of the dermal bones, the quadrato-jugal of the *Stegocephali* would arise. This possible origin of the bone finds some support in Gaupp's (No. 17, p. 104) serial grouping of the three bones called by him "*Squamosum*," "*Paraquadratum*," and "*Quadrato-maxillare*," the *Squamosum* being the upper and the *Quadrato-maxillare* the lower element of the series. The fact that the preoperculum of fishes is formed by the fusion of several components, more or less numerous according to the number of sense organs that develop in the line, and the further fact that the organs of the line in *Amia* first appear at or near the middle point of the line, and develop or differentiate from there in both directions, may offer some explanation of the apparent disappearance, to which Gaupp calls attention, in certain animals or classes of animals, of one or the other of the three elements of this series.

The quadrato-jugal of the *Stegocephali* is considered by Baur (No. 5) as the probable homologue of the suboperculum of fishes.

The jugal of reptiles varies greatly in its relations to the adjacent bones. In *Dimetrodon* (No. 6) and in the crocodile (No. 17, Fig. 5) it lies wholly posterior to the maxillary bone, as it does in *Trematosaurus*. In *Branchiosaurus* (No. 16, p. 68, Fig. 30) it has nearly the same length as the maxillary and quadrato-jugal together, and lies immediately superior to them and parallel to them. In *Chelydosaurus* (No. 16, Bd. II, p. 21) it is separated from the lower edge of the orbit by an anterior prolongation of the postorbital bone, and is completely fused with the lachrymal. In this animal there are thus three parallel lines of bones below the orbit: the postorbital, the jugal and supratemporal, and the maxillary and quadrato-jugal.

If the jugal bone of reptiles and the maxillary bone of *Polypterus* are, as here suggested, developed in part, in connection with homologues of the horizontal cheek line and the sub-



orbital canal line of the lateral-line organs of *Amia*, and fused, in *Polypterus*, with the maxillary and supramaxillary bones, the zygomatic arch of man must be this chain of sense-organ bones fused, in part, and in part articulated with each other and with the proximal part of the preoperculum, and the latter bone fused with the squamosal. The persistence of this chain of bones, and the disappearance of the long preopercular connection between the squamosal and the lower jaw, so characteristic of fishes, would be naturally associated with the disappearance of a suspensorial apparatus.

The zygomatic process of the temporal bone of man is, then, a part of the preoperculum of fishes; and the malar bone is certain of the infraorbital bones, fused, perhaps, with other elements, such as the supramaxillary, or a post-suborbital element not found in *Amia*. The occasional division of the malar bone into two parts by a horizontal suture (No. 32, Vol. II, Pt. I, p. 56) may be due to this origin of the bone in connection with two lines of sense organs, or, possibly, to the fusion of bones of sense-organ origin with others developed in connection with teeth-bearing plates. The abnormal formation, in the skull described by Török (No. 43), of a distinct suborbital arch, consisting of a process of the malar bone and two separate and independent bones, is apparently due to a still further separation of these same two elements.<sup>1</sup>

<sup>1</sup> Maggi, in a recent publication ("Autres résultats de recherches morphologiques sur des os crâniens et sur des fontanelles de l'homme et d'autres mammifères," *Arch. Ital. de Biol.*, Tome XXX, Fasc. II, 27 Dec., 1898), makes the following statement: "Le centre d'ossification de la squame proprement dite ou de la portion squameuse du temporal qui se manifeste un peu au-dessus de la place correspondant à la base de son apophyse zygomatique, est homologue au pré-operculaire du *Polypterus*; le centre d'ossification de l'apophyse zygomatique du temporal est homologue au sub-operculaire du *Polypterus*; le centre d'ossification de l'épitympanique de Rambaud et Renault, ou serriale d'Et. Geoffroy Saint-Hilaire, est homologue à l'operculaire du *Polypterus*." The homologue here proposed for the preoperculum of *Polypterus* differs radically from that proposed by me, in that Maggi finds it in a part of the squamosal, at the base of the zygomatic process, instead of in that process itself. In the same publication Maggi states that the postorbital bones are found in the human infant as "noyaux d'ossification . . . placés sur le côté antérieur de l'extrémité triangulaire de la grande aile du sphénoïde." According to my determination they would be found in the frontal process of the malar bone. As Maggi himself says ("Post-frontale nei Mammiferi," *Rendic. R. Istit. Lomb. Sc. Lett.* (2) V. 30, Fasc. 9)

The preorbital ossification (*PRE*) is of primary origin, and contains, in *Amia*, no canal organ component. It may, however, receive membranous additions to its dorsal surface, as I have already pointed out (No. 1, p. 478). A strong, broad, fibrous band arises from its lateral surface, and is inserted on the superior surface of the autopalatine. This band holds the palato-quadrate arch up against an articular ridge on the ventro-lateral edge of the chondrocranium, immediately ventral to the preorbital ossification. The ossification itself takes no direct part in the articulation of the palato-quadrate with the cranium. It gives attachment, on its postero-inferior surface, to the third division and part of the fourth division of the levator maxillae superioris muscle.

A wholly separate, and entirely dermal, prefrontal bone is described in *Amia* by Bridge (No. 7, p. 615), but I have been wholly unable to find it. I also find no such bone described, as such, in any other fish excepting *Clarias*. In the latter fish Pollard says (No. 31, p. 527) there is a dermal prefrontal, lying between the nasal and frontal bones, and that it is traversed by the main supraorbital lateral canal. This course of the canal is confirmed by Collinge (No. 12, p. 275), who calls the bone it traverses the lateral ethmoid. It contains, according to Pollard, no sense organ of the canal line by which it is traversed, and hence cannot be developed in the same direct connection with the line that the frontal and nasal bones are. What its representative in *Amia* and other fishes may be is in no way evident.

The septomaxillary (*SMX*) is a primary ossification of the chondrocranium lying ventral to the anterior end of the nasal sac, and forming the antero-lateral part of the floor of the nasal pit (No. 7, p. 615; No. 3, Figs. 8, 10). It is said by Bridge to be an ossification peculiar to *Amia* amongst ganoids, and the name septomaxillary was given to it by him because of its supposed homology with a bone described by Parker in the nasal capsule of the frog, and called by Parker in that animal

that the postfrontal is found, in man and mammals, either as a separate bone lying between the frontal process of the malar and the external angular process of the frontal, or fused with one or the other of those bones, it would seem much more natural to look for the postorbitals in the malar bone than in any connection with the wing of the sphenoid.



the septomaxillary. This bone in the frog is said by Parker to be something "more than half a tube, lining the front of the nasal passage and sending down a curled process, which can be seen from the palate" (No. 25, p. 175). On the dorsal surface of the skull the bone is said by Parker to be seen "as a little grain of bone jammed in between the nasal process of the premaxillary and the facial plate of the maxillary, in front of the outer nostril." Sagemehl, although accepting in the fishes described by him the name given by Bridge to the bone in *Amia*, questions its homology with the similarly named bone of the frog (No. 35, p. 204). Both he and Bridge assert the homology of the bone in *Amia* with a well-known bone in *Esox*, that bone being bone 3 of Huxley's descriptions (No. 21, p. 133).

The septomaxillary of *Amia* is an ossification that begins on the outer surface of the cartilage of the chondrocranium, and extends into it from the point, or line, where the chondrocranium, in larvae, gives articulation to the anterior end of the maxillary bone. In the adult fish this articular surface is formed by the straight anterior edge of the septomaxillary, the bone presenting posteriorly, toward and in the cartilage, a rounded and roughly semicircular outline. The dorsal surface of the bone is covered wholly, or in part, by the posterior process of the premaxillary, which lies directly upon it. The ventral surface of the lateral edge of the bone forms the anterior end of a low, longitudinal, condylar eminence, which extends backward along the ventral surface of the lateral edge of the antorbital process of the chondrocranium, and gives articulation to the palatoquadrate arch.

In the Cyprinidae (No. 37, pp. 510, 582) the long and single palatoquadrate articular eminence of *Amia* is represented by two condylar processes, the anterior of which is said by Sagemehl to be entirely of cartilage in certain species, and partly ossified in others. The ossification, where this process is partly ossified, is considered by Sagemehl as the homologue of the septomaxillary of *Amia*, and its articular surface is said to be always capped with cartilage. The process, although said to be a "Vorsprung" of the vomer, has, in the figures, decidedly

the appearance of being simply an articular process of the chondrocranium, and not a part of the vomer. The septomaxillary in these fishes thus seems to be an ossification formed in an articular process of the chondrocranium, to strengthen it, and not, as in *Amia*, an ossification formed directly at the articular surface of the process. If, however, the hind end of the articular ridge of *Amia* were to develop into a lateral process, and the articulation of the maxillary to be transferred from the bone to the cartilaginous point of this process, the resemblance would be exceedingly striking.

In *Esox*, where there are two articular heads for the palatoquadrate, as in the Cyprinidae, the septomaxillary, or bone 3 of Huxley, forms the anterior one of the two. The bone, as in *Amia*, is not capped with cartilage, and it extends into the cartilage from the articular surface as a center.

In the Characinidae, where there is no septomaxillary, the palatine bone either articulates at its front end with, or is loosely attached by fibrous or ligamentous bands to, the under surface of the ethmoidal region of the chondrocranium (No. 36, pp. 93, 95).

In *Gadus aeglefinus*, where there is also no septomaxillary, the palatine bone is said by Brooks (No. 8, p. 174) to articulate, at about the middle of its length, with "a cartilaginous eminence on the junction of the prefrontal and vomer," the eminence, in the figures, seeming to lie between the ethmoid and vomer as much as, or even more than, between the latter bone and the prefrontal. This ethmoidal articulation of the palatine seems, from the descriptions, to be the only one that the palatoquadrate of *Gadus* has with the skull, the posterior, antorbital articulation of the arch being replaced by ligamentous attachment only.

In *Polypterus*, where also no septomaxillary bone can be identified from the descriptions, the palatine bone, as identified by Traquair (No. 44, pp. 175, 177), is reduced to a small ossicle which articulates with the "lower margin of the projecting anterior, inferior angle of the prefrontal." At this point Polard shows also a ligamentous connection of the palatine with the ventral surface of the ethmoidal cartilage (No. 31, Fig. 33).

The anterior palatal articulation of *Amia* thus seems to be wanting in *Polypterus*. There is, however, another, more anterior connection, rather than articulation, of the palato-quadrato with the skull. It is effected through the intermediation of a bone considered by Traquair as the homologue of the vomer both of *Amphibia* and of fishes. This so-called vomer bone, of which there are two in *Polypterus*, one on each side of the head, lies "in contact above with the median ethmoid, with the premaxillary, and with the cartilaginous floor of the nasal chamber; by the greater part of its outer edge it articulates by suture with the maxillary bone, while its posterior extremity is articulated with the ectopterygoid, coming also into close contiguity with the palate-bone and prefrontal." The bone is said by Traquair to have been considered by Agassiz as a part of the superior maxillary, and by Müller as a part of the palate bone. It certainly seems to be the piscine dermo-palatine rather than the vomer, and I shall refer to it again in treating of the latter bone.

It may here be stated that the bone identified by Traquair as the palatine of *Polypterus* was apparently described before him by Erdl, and considered by Erdl as the probable homologue of the anterior "Gaumenbein" of the carp (No. 13, p. 244).

The septomaxillary element of the skull of ganoids and teleosts thus seems to be developed in connection with an anterior ethmoidal articulation of the palato-quadrato with a ventrolateral portion of the chondrocranium. Moreover, both in *Amia* and in *Esox*, the only fishes so far described in which the articular surface of the bone is found uncapped by cartilage — that is, as an ossification extending into the cartilage from the articular surface with which it is associated — it is found associated with a strictly dermal ethmoid, and not with a primary one. This would seem to indicate that it may have its homologue in some part of the primary ethmoid bone of those fishes in which that bone is found, and hence, according to generally accepted views, in the vertical plate of the ethmoid bone of man. The relations of the two septomaxillary bones of *Amia* to adjacent bones of the region suggest, however, that they



may possibly become the vomer bone of man, as will again be referred to below.

The premaxillary (*PMX*) of *Amia* consists, as Bridge has stated (No. 7, p. 610), of two parts, — a thickened, anterior, marginal portion, and a spoon-shaped, posterior portion, the latter being called by Bridge the ascending portion of the bone. The marginal portion bears several large teeth, usually eight in number, and has a roughened median end where it adjoins, and is bound by fibrous tissue to, its fellow of the opposite side. The posterior portion of the bone arises, approximately, from the mesial two-thirds of the marginal portion, and it is perforated in the center for the passage of the olfactory nerve. It lies directly upon the floor and sides of the nasal pit, those surfaces of the pit being formed by the dorsal surface of the septo-maxillary and the dorsal surface of the chondrocranium around and posterior to that bone. The hind end of the bone becomes thin and extends backward under the frontal, in some specimens as far as, or even slightly beyond, the hind end of the preorbital ossification. This part of the bone lies ventral to that thin anterior process that arises from the mesial end of the anterior edge of the frontal, but dorsal to the similar process that arises from the lateral end of the anterior edge of the bone.

At the extreme antero-mesial end of the large olfactory perforation of the posterior process of the premaxillary there was, in all the specimens first examined, a partly formed foramen, which transmitted, from below upward, a terminal branch of the ramus palatinus anterior facialis (No. 3, p. 620, and Figs. 8, 10, and 21). This nerve, immediately after issuing from its foramen, anastomosed with terminal branches of the ramus maxillaris superior trigemini, and one or more branches of the plexus so formed, accompanied by vessels, immediately entered one or more small foramina which lay immediately in front of the first-mentioned foramen, and led into canals in the premaxillary bone. In the specimen from which the present drawings are made, a relatively young one, the partly formed, first-mentioned foramen was found as a fully formed one (*paffr.*, Figs. 2-4), and the canal leading downward from it

through the premaxillary entered at once the vomer without piercing at all the cartilage that forms the floor of the olfactory pit, as it did in the first specimens examined. The canal in this specimen thus lay entirely anterior to the cartilage of the chondrocranium, and the anterior end of the palatine nerve ran upward in front of the anterior edge of the chondrocranium, as it did also in all the larvae examined. The nerve, also, in this specimen, did not reach the level of the upper surface of the premaxillary, but appeared on the dorsal surface of that bone at the hind end of a short groove or pit, at the anterior end of which another foramen, corresponding to the separate anterior foramina of the first specimens examined, led into the premaxillary. The enclosing of the distal end of the anterior branch of the palatine nerve in a cartilaginous canal thus seems to be a secondary process belonging to post-larval stages.

The mesial end of the anterior, alveolar portion of the premaxillary lies directly beneath and gives support to the anterolateral end of the ethmoid, the two bones being, in one large specimen, firmly connected by partial anchylosis. The anterior end of the antorbital rests upon the dorsal surface of the premaxillary immediately lateral to the lateral end of the ethmoid. The nasal lies dorsal to the spoon-shaped portion of the bone, but separated from it by the nasal sac. The posterior portion of the mesial half of the ventral surface of the anterior part of the bone is roughened, and rests directly upon a corresponding portion of the dorsal surface of the anterior end of the vomer, the two surfaces adhering closely to each other and seeming to be in process of anchylosis. Lateral to this roughened surface there is, on the ventral surface of the bone, a sharply depressed, almost pit-like region, which extends to the lateral edge of the bone. It forms the anterior and dorsal surfaces of an articular pocket which receives the anterior articular end of the maxillary. The anterior edge of the septomaxillary forms the posterior surface of this pocket, and a slight transverse groove on the dorsal surface of the vomer its ventral surface.

In *Polypterus* the premaxillary lies, according to Traquair (No. 44, Figs. 1 and 5), external to the ethmoid bone, not only on the dorsal surface of the skull, but also on its ventral surface.



According to Pollard's figures (No. 30, Figs. 12 and 15), it lies ventral to the anterior end of the chondrocranium, but not external to the ethmoid either on the dorsal or the ventral surfaces of the skull. The hind ends of the ventral plates of the two bones, in Pollard's figures, approach the anterior end of a median, dermal bone, called by him the subrostral (No. 30, p. 412). This latter bone bears teeth and is said to lie ventral to the anterior end of the parasphenoid, between the posterior parts of the adjoining antero-mesial ends of the so-called vomers. If the ventral plates of Pollard's premaxillary bones should fuse with the subrostral a ventral plate would be formed the exact equivalent of the ventral plates of the premaxillaries of Traquair's descriptions, fused in the median line with each other.

The single subrostral of Pollard thus seems to be the homologue of the vomers of *Amia* fused with each other and greatly reduced in size. In Traquair's specimen, as in *Amia*, these two halves of the subrostral seem not to have fused with each other. They have, however, completely fused with the premaxillaries, instead of only partly so, as in *Amia*.

The premaxillary bone of *Polypterus*, as shown by Traquair, has two ascending processes—a small one, mesial to the anterior nasal aperture, and another, much larger one, lateral to that aperture and lateral also to the posterior nasal aperture.

In teleosts the ascending process of the premaxillary, which is, according to Sagemehl, rarely wanting (No. 36, p. 99), arises from the mesial end of the horizontal alveolar portion of the bone, lies mesial to the anterior nasal aperture, or wholly in front of it, and is bound directly or indirectly to the anterior end of the skull. Where it is bound indirectly to the skull by the intermediation of a separate cartilaginous rostrale, that element lies external to the ethmoid bone, or external to the ethmoidal region of the chondrocranium. In the Cyprinidae, where the ascending process is small, it is bound to the anterior end of the skull by a fibrous band, in which there is almost always a small median bone which Sagemehl considers as the ossified rostrale (No. 37, p. 584).

In Urodela (No. 27) the ascending process of the premaxil-

lary lies mesial to the nasal capsule, and may extend backward beyond the anterior end of the frontal, always lying, in such cases, superficial to that bone. In certain of Parker's figures it is shown internal to the nasal bone (Pl. XIX, Fig. 4); in others it seems to lie external to that bone (Pl. XV, Fig. 5); and in still others (Pl. XX, Figs. 1 and 3) it seems to be completely or incompletely separated into two parts, the posterior of which lies external to the frontal. It always lies mesial to the nasal bone.

Is, then, the so-called ascending process of the premaxillary of *Amia*, which lies internal to the ethmoid and nasal bones and ventral to the nasal sac of the fish, the homologue of the similarly named process of teleosts and urodeles which always lies external to the ethmoid, and either anterior to, or mesial to, the nasal bones and nasal capsules? It seems to me most improbable, and I look for the homologue of the ascending process of teleosts and urodeles either in some small part of the posterior process of *Amia* fused with the ethmoid bone of that fish, or in the latter bone alone. This latter possibility for a part of the ascending process of *Siren lacertina* seems to have already been suggested by Wiedersheim (quoted No. 16, Bd. I, p. 109). The small median bone considered by Sagemehl, in the Cyprinidae, as the ossified rostrale is then simply the ethmoid bone of *Amia* greatly reduced. The large, lateral ascending process of *Polypterus* I consider, as already stated, as the antorbital bone of *Amia* fused with the premaxillary.

What, then, is the posterior process of the premaxillary bone of *Amia*? Its general shape, its relation to the nasal sac, and its perforation by the olfactory nerve seem to me to indicate, almost conclusively, that it is a bone developed in relation to a lateral sense organ that has given origin to the sensory epithelium of the nose, just as the different sections of the several canal bones are developed in relation to the sense organs they are destined to protect or enclose. The nasal sense-organ bone has simply retained its larval form, and fused with the tooth-bearing premaxillary plate which lies immediately in front of it. The piercing of the premaxillary bone by the ramus palatinus anterior facialis, simply to reënter the bone again from

its dorsal surface, is then naturally explained. It has been caught between the two parts of the bone before they fused.

In *Gymnarchus niloticus* the nasal sense-organ bone is still found as a separate bone, and is described by Erdl (No. 13) as bone No. 4. It forms, in *Gymnarchus*, a direct, anterior continuation of the infraorbital chain of bones, lying intercalated between the most anterior of those bones and the premaxillary bone. The latter bone has a short palatal plate, but no other process of any kind. The infraorbital bones are said by Erdl to be half canals, and they undoubtedly partly enclose an infraorbital lateral canal, although that canal is not described. The nose is thus, apparently, in the adult of *Gymnarchus niloticus*, simply the most anterior organ of the main infraorbital, lateral line, just as it seems in a measure to be in larvae of *Amia* (No. 1, pp. 509, 531, 537) and of *Necturus* (No. 29, p. 492; Pl. XXXVIII, Fig. 1). Bone 3 of *Gymnarchus* is too evidently the nasal bone to need any comment. Bone 7, which is called by Erdl the nasal, seems to be a purely dermal, piscine ethmoid.

The bone that forms in connection with the nasal lateral sense organ, in fishes other than *Amia* and *Gymnarchus*, I cannot recognize as a separate bone in any descriptions at my disposal,<sup>1</sup> unless it be, as already stated, in bone 2 of *Esox*. The

<sup>1</sup> Since my manuscript was sent to the publishers I have received three of Broom's Works: "On the Homology of the Palatine Process of the Mammalian Premaxillary," *Proc. Linn. Soc., N. S. Wales*, Vol. X, Pt. III, 1895; "On the Occurrence of an Apparently Distinct Prevomer in *Gomphognathus*," *Journ. Anat. & Phys.*, Vol. XXXI, N.S., Vol. XI, 1897; and "On an Apparently Hitherto Undescribed Nasal-Floor Bone in the Hairy Armadillo," *Journ. Anat. & Phys.*, Vol. XXXI, N.S., Vol. XI, 1897. In the first of these three publications Broom states his conclusion that the palatine process of the premaxillary of mammals is the homologue of the dumb-bell-shaped bone of *Ornithorhynchus*, of the vomer of *Reptilia*, etc., and he proposes for all these bones the name prevomer. He states that J. T. Wilson before him had recognized the vomerine character of the dumb-bell-shaped bone, and on page 483 of his own work he briefly summarizes the main arguments advanced by that author in support of his conclusions. If they be consulted, it will readily be seen that they all apply with equal force to the posterior process of the premaxillary of *Amia*, which bone is thus possibly the piscine homologue of the dumb-bell-shaped bone of *Ornithorhynchus*, and in that case, if Broom is correct, the homologue also of the palatine process of the premaxillary bone. It is, however, certainly not the homologue of the so-called vomers of *Polypterus*, which are generally considered as the homologues of the vomers of *Amphibia*, if



relation of that bone to the septomaxillary and its turned-down lateral edge suggest the possibility of its being the homologue of the posterior process of the premaxillary of *Amia*, although in all other respects it much more resembles the ethmoid. In certain of the Cyprinidae the bone seems to have fused with the preorbital ossification instead of with the premaxillary, as the conditions described by Sagemehl (No. 37, p. 566) in the genera *Labeo* and *Osteochilus*, in particular, strongly indicate. In the frog the bone may be represented in the septomaxillary. If it exists in man, it must certainly have its homologue in that part of the ethmoid that binds the lateral masses of the bone to the vertical plate; that is, in some part of the cribiform plate. The fact that the nasal sac is, in selachians (No. 47, p. 106) and in certain cyprinoids (No. 37, p. 568), separated from the cranial cavity by a membranous lamina cribrosa strongly supports this supposition. If this be so, the anterior end of the bone in *Amia* is certainly the homologue of the posterior end of the plate in man; and if the nasal apertures of animals, one or both, lie primarily on the ventral surface of the head, as Wiedersheim states (No. 46, p. 304), the position of the bone in *Amia* and *Gymnarchus* must necessarily be a secondary one. Whether the internal nasal aperture of man is of secondary origin, as Wiedersheim states, or is the homologue of one of the external apertures of *Amia*, would make no difference in this conclusion. The crista galli of man would, in either case, seem to be represented in *Amia* by the hind end of the cartilaginous wall that separates the olfactory canals and looks backward into the anterior extension of the cranial cavity.

If these several homologies are correct, the anterior nasal spine of the superior maxillary bone of man is naturally the ascending process of the premaxillary bone of teleosts and urodeles — the separate ethmoid bone of *Amia* — and the nasal

not also of those of *Reptilia*. If this process of *Amia* is here correctly identified, the vomers of *Amia* must be either the prepalatine elements of Sutton's descriptions, or those palatal plates of the premaxillaries of *Gomphognathus* that are said by Broom (*loc. cit.*, p. 278) to meet in the middle line behind the median choana. The "apparently hitherto undescribed nasal-door bone" would then seem certainly to be the septomaxillary of *Amia* and the parasphenoid of that fish the vomer of mammals, as Sutton states.

process of the bone of man is the antorbital bone of *Amia* fused either with the maxillary or premaxillary of that fish.

The so-called vomer (*VO*) of *Amia* is found as two separate bones, one on each side of the head, instead of as a single median one, as in most fishes (No. 7, p. 610; No. 35, p. 186). In the specimen examined by Bridge the anterior thirds of the two bones are said to have been suturally united with each other. The dorsal surface of the anterior end of each bone rests against the ventral surface of the anterior, alveolar portion of the premaxillary, and adheres closely to that surface, as already stated. Posterior to this portion of the premaxillary the vomer lies ventral to the anterior, articular end of the maxillary, then against the ventral surface of the septomaxillary, and, towards its posterior end, against the ventral surface of the anterior portion of the parasphenoid. Between the anterior ends of the two bones a small part of the anterior end of the chondrocranium is exposed on the ventral surface of the skull.

These relations of the vomers of *Amia* to the premaxillary and maxillary bones thus seem to indicate that their homologues should be looked for in some part of the palate plate of the superior maxillary bone of higher vertebrates, and not in the vomer bone. That a bone that lies ventral to the horizontal part of the maxillary in one vertebrate should lie dorsal to it in others seems improbable.

In *Polypterus* there are, as already stated, two bones which, together, are considered by Traquair as the homologue not only of the vomer bone of other fishes, but also of those of all other vertebrates. They are said by him to have been considered by Agassiz as parts of the superior maxillary bones, and by Müller as parts of the palate bones. Van Wijhe (No. 47) and Pollard (No. 30) both accept Traquair's identification of these bones, and hence the necessary consequence that there is no separate dermo-palatine in *Polypterus*. In van Wijhe's opinion (No. 47, p. 253), the latter bone is fused with the ectopterygoid, and forms the anterior end of the bone so named. This conclusion is based on the supposition that the dermal component of a bone must always be found in close relation to its cartilage



component, a supposition which, according to Gaupp, is incorrect (No. 17, p. 82).

Between the anterior ends of the so-called vomers of *Polypterus*, Pollard found and described, as already stated, a small, median, impair bone, which he was at first inclined to consider as the vomer, though it is said not to be the homologue of the two so-called vomer bones of *Amphibia*, which he identified as the dermo-palatine. Later he concludes that this impair bone is a new bone "which only doubtfully reappears again in the animal kingdom," and he accordingly gives it a new name, calling it the dermal subrostral. As already stated, I consider this median, subrostral bone of Pollard as the probable homologue of the vomers of *Amia* fused with each other, and I am inclined to think that the single, separate bone of Pollard's specimen is found in Traquair's specimen, which was possibly a different variety of *Polypterus*, as two separate parts, each of which is fused with the premaxillary of its own side of the head.

If this be so, the so-called vomers of *Polypterus* must necessarily be the homologues of the palatine bones of other fishes, as Müller is said to have asserted, or of the dermo-palatines, as Pollard (No. 30, p. 412) has suggested. I consider them the homologues of the dermo-palatines, a conclusion which entails, according to Pollard, the further conclusion that the two vomer bones of urodeles and the single vomer bone of mammals are the homologues of the dermo-palatines of fishes, and not of the vomer bone or bones. This conclusion, in so far as it relates to urodeles and other amphibians, I fully accept.

According to Sutton (No. 42), the so-called vomer bone or bones of fishes and amphibians are represented in man and mammals by the prepalatine part of the hard palate, that part of the palate ossifying from a distinct and separate center of the superior maxillary bone. This statement presupposes the homology of the vomer bones of fishes and amphibians, which, as stated above, I am strongly inclined to doubt. It seems to me much more probable that the so-called vomer bones of *Polypterus* and *Amphibia* are represented in the horizontal plate of the palate bone of man and mammals, and not in the

palate plate of the superior maxillary; and that the vomer bone or bones of fishes, other than *Polypterus*, are represented either in that horizontal piece which, according to Sutton, projects backward, in man, from the mesial surface of the premaxillary part of the superior maxillary bone, and forms the inner boundary of the anterior palatine canal; or in some part, but not the whole, of Sutton's prepalatine part of the hard palate. The primary relation that the vomer is said to acquire to the chondrocranium in certain fishes (No. 36, p. 40) is, then, simply repeated in the similar relation said by Sutton (No. 42, p. 569) to be acquired by a part of the premaxillary bone of man.

The vomer bone of fishes not being the homologue of the vomer of man, it remains to seek in the former animals the homologue of the latter bone. According to Sutton, it is to be found in the anterior end of the parasphenoid. According to Pollard's supposition, it would be represented by the dermo-palatine. According to Erdl (No. 13), it is represented by the so-called ethmoid. But for the fact that the septomaxillary of *Amia* is preformed in cartilage, and not in membrane, it would seem to fulfill even better than any of these bones the conditions of a mammalian vomer. It rests directly upon the piscine vomer, the probable homologue of a part of the palate plate of the superior maxillary bone of man; and it articulates with the palatine, thus coming into relations both with the auto- and the dermo-components of that bone, the latter of which is probably the homologue of the horizontal plate of the palate bone of man. If the nasal septum were to become gradually thinner, the septomaxillary would, if retained, naturally extend upward and backward, in the septum, toward the ventral edge of the primary ethmoid, which bone is generally considered as the homologue of the vertical plate of the ethmoid bone of man, and which, in teleosts, ossifies downward from the dorsal surface of the snout.

Bearing the several homologies here indicated in mind, imagine the snout of *Amia* pushed backward between and below the eyes, under the anterior end of the cerebral cavity, where it is found in man. Imagine, also, the cartilaginous floor of the nasal cavities absorbed; the vomer reduced in length

because of that absorption of its underlying support; the dermo-palatines separated from the ectopterygoids and brought together in the middle line of the head; and the auto-palatine separated from the dermo-palatine, excepting along the lateral edge of that bone, and immovably, instead of movably, articulated with the preorbital ossification.

The posterior surface of the preorbital ossification would then form part of the inner wall of the orbit, as its supposed homologue, the lateral mass of the ethmoid, does in man. Between it and the frontal would be found the openings of the internal orbital canals, which canals in man transmit the nasal nerve and nasal vessels, and in fishes, nasal vessels, and, where it is found, the ramus ophthalmicus profundus.

The auto-palatine would present a free, orbital surface between the preorbital ossification and the pterygoid bone, with both of which it would be articulated; and it would lie immediately internal to and adjacent to the maxillary.

The lachrymal, if slightly detached from the suborbital bones, — or, better still, a dermal prefrontal, if it existed, as Bridge says it does, — would lie, as the lachrymal does in man, between the preorbital ossification posteriorly, the frontal dorsally, and the antorbital anteriorly, the latter bone being the homologue of the nasal process of the superior maxillary bone of man.

The posterior nasal aperture would lie approximately between the antorbital bone and the lachrymal or prefrontal, as the case may be; and the tube leading from the aperture to the nasal sac, or that tube plus a part of the canal that, in embryos, connects the tube with the infraorbital lateral canal, would correspond closely in its relations to the adjoining bones to the lachrymal groove of man. If, then, the naso-lachrymal canal of higher vertebrates is developed, as Wiedersheim suggests (No. 47, p. 313), from some part of the lateral-line canals of fishes, the posterior nasal canal of *Amia*, or its embryonic connection with the anterior end of the suborbital lateral canal, must be that part. In support of this, it is important to note, that in all those animals in which the naso-lachrymal canal is found there is but one external nasal aperture on each side of the head; and that the naso-lachrymal canal opens into the nasal



fossa, near that external aperture, not only in man (No. 32), but also in certain, if not in all, animals in which it is found (No. 40).

The external nasal aperture in the rabbit, cat, and man, is, according to Hochstetter (Nos. 19, 20), the only primary opening of the nasal pit, the internal aperture being of secondary origin. The single external aperture of these vertebrates must, if this be true, be the homologue of the two apertures combined of *Amia*. From Hochstetter's descriptions I am, however, inclined to think that the nasal pit, in the animals investigated by him, simply closes irregularly by the partial coalescence of its lips at the distal end of the primary nasal depression, instead of at its middle point. The distal end of the nasal depression, the future internal nasal aperture, is thus simply temporarily closed, and then reopened after having been enclosed in the mouth cavity. Such being the case, the internal nasal aperture of man would be the homologue of the anterior aperture of *Amia*, the external aperture the homologue of the posterior aperture of *Amia*, and the lachrymal canal and pore a part of the suborbital lateral canal. Seydel's statement (No. 40) that there is always, in the nasal sac of *Amphibia*, a line of non-sensory tissue extending along the floor of the nasal fossa, from the external to the internal apertures, strongly supports this supposition, the non-sensory line representing, naturally, the line where the lips of the primary nasal depression have fused to form a canal. Further support of this is found in the development of *Ceratodus*. In this fish (No. 39, pp. 42-44) the nasal pit lies at first wholly outside the mouth cavity, as in *Amia*. A nasal groove is then formed, the anterior end of which is first enclosed, secondarily, in the mouth cavity; and then the entire groove is so enclosed. The two lips of the groove then approach and fuse, and the two nasal apertures are formed, exactly as in *Amia*. It is, moreover, to be noted that, although both apertures lie apparently inside the mouth cavity, in the adult, the anterior one of the two lies anterior to the upper edge of the mouth, as defined by Semon (No. 39, p. 45), — that is, in reality, outside the mouth, in the upper lip. This indicates that the nasal sac of higher

vertebrates must be entirely enclosed before the maxillary and premaxillary bones begin to develop, the premaxillary developing, after the enclosure of the sac, in the mesial lip of the pit, the maxillary in the lateral one. The incisor foramen is then simply a persistent part of the primary nasal groove.

The infraorbital bones of *Amia*, or, if the prefrontal of fishes is the homologue of the lachrymal of man, the infraorbital bones with the lachrymal, would become the malar bone.

The septomaxillary, if it did not disappear or become part of the superior maxillary bone, would be forced into the nasal septum and become the vomer bone. Or, if the septomaxillary does not become a vomer, the anterior end of the parasphenoid might become that bone, as Sutton concludes.

The anterior branch of the ramus palatinus facialis, which lies, in larvae of *Amia*, directly against the ventral surface of the chondrocranium, would, as the floor of the nasal fossa disappeared, enter the fossa and lie along the external surface of the septomaxillary or the corresponding surface of the parasphenoid, according as one or the other of these bones becomes the vomer; just as the naso-palatine nerve in man does along the external surface of the vomer.

The posterior branch of the ramus palatinus facialis of *Amia* (No. 3, p. 619) runs outward and downward, inferior to the persisting cartilaginous part of the palato-quadrate arch, inferior to the auto-palatine, superior to all the dermal bones of the palatine arch, and then ventral to the horizontal articular end of the maxillary bone, and ventral to the premaxillary. It would thus lie in front of the assumed future horizontal plate of the palate bone and behind and then ventral to the future horizontal plate of the superior maxillary bone, just as the large anterior palatine nerve does in man. It would, however, lie internal to the auto-palatine, the future vertical plate of the palate bone, exactly the contrary to what is found in man. The nerve in *Amia*, however, varies greatly in its relation to the auto-palatine, sometimes piercing that bone and issuing on its dorsal surface, and, if van Wijhe's figure is correct (No. 47, Fig. 11), lying sometimes wholly dorsal to it. The nerve would in these latter cases lie between the future vertical plate of the palate bone and the



superior maxillary bone, exactly in the position of the posterior palatine canal of man.

Near the articular end of the maxillary the posterior palatine nerve of *Amia* forms an anastomosis with the maxillary branch of the maxillaris superior trigemini. A similar anastomosis in cyprinoids is considered by Sagemehl (No. 37, p. 558) as unquestionably the representative in fishes of the spheno-palatine ganglion of higher animals. This ganglion in man (No. 32, Vol. II, Pt. II, p. 24) lies in the spheno-maxillary fossa, between the sphenoid bone posteriorly, the superior maxillary bone anteriorly, and the vertical plate of the palate bone internally. From it both the naso-palatine and large palatine nerves arise. The anastomosis between the posterior palatine and superior maxillary nerves of *Amia* must, therefore, be pushed backward by the shortening of the snout to a position slightly in front of the cartilaginous, anterior clinoid-wall of the fish, — a position which would correspond closely, in its relation to the neighboring bones, to the spheno-maxillary fossa of man. As there are no ganglionic cells at the anastomosis of the two nerves in *Amia*, those cells must migrate into it from somewhere, possibly, as I was led to suggest in my earlier work (No. 2), though it seems to me now not probably, from the mass of cells, apparently sympathetic, found on nerve "n" of Pinkus.

That part of the superior maxillary nerve of *Amia* that lies distal to its anastomosis with the palatinus facialis, and runs forward and inward dorsal to the horizontal part of the maxillary bone, would correspond approximately, in position, to the inferior nasal branch of the large palatine nerve of man. The naso-palatine anastomosis with the great palatine nerve would have its approximate counterpart in *Amia*, the canals traversed by the anterior ends of the anterior palatine nerves of the fish being the foramina of Scarpa.

The maxillary branch of the superior maxillary nerve of *Amia*, issuing between the inferior margin of the lachrymal, or first suborbital bones, and the superior margin of the maxillary, would naturally give origin to the infraorbital canal of man.

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## DESCRIPTION OF PLATE XXXIII.

## INDEX LETTERS.

<i>ana</i>	Anterior nasal aperture, or nasal tube.	<i>pc</i>	Palatine canal.
<i>ANT</i>	Antorbital.	<i>PMX</i>	Premaxillary.
<i>AUP</i>	Auto-palatine.	<i>pna</i>	Posterior nasal aperture.
<i>DP</i>	Dermo-palatine.	<i>POR</i> <sup>1</sup>	First postorbital.
<i>ECP</i>	Ectopterygoid.	<i>POR</i> <sup>2</sup>	Second postorbital.
<i>ENP</i>	Entopterygoid.	<i>ppc</i>	Canal for ramus palatinus posterior facialis between the auto- and dermo-palatine bones.
<i>ETH</i>	Ethmoid.	<i>PRE</i>	Preorbital ossification; prefrontal of Sagemehl.
<i>FR</i>	Frontal.	<i>PS</i>	Parasphenoid.
<i>JG</i>	Jugal, or supramaxillary.	<i>PSF</i>	Postfrontal.
<i>LA</i>	Lachrymal.	<i>PST</i>	Postorbital ossification.
<i>MX</i>	Maxillary.	<i>SOR</i> <sup>1</sup>	First suborbital.
<i>NA</i>	Nasal.	<i>SOR</i> <sup>2</sup>	Second suborbital.
<i>olfr</i>	Olfactory foramen.	<i>SMX</i>	Septomaxillary.
<i>OS</i>	Orbitosphenoid.	<i>VO</i>	Vomer.
<i>pafr</i>	Foramen for ramus palatinus anterior facialis.		

## EXPLANATION OF PLATE.

FIG. 1. Side view of anterior half of skull of adult *Amia*, with the fibrous and ligamentous tissues connecting the bones left in place.  $\times 2$ .

FIG. 2. Same as above, with the nasal, antorbital, lachrymal, and suborbital bones removed.  $\times 2$ .

FIG. 3. Top view of same skull. On the right side the ethmoid, nasal, and antorbital bones have been removed. On the left side all the canal bones have been removed.  $\times 2$ .

FIG. 4. Same as Fig. 3, with the premaxillary bone and the palato-quadrate arch removed on the left side.  $\times 2$ .

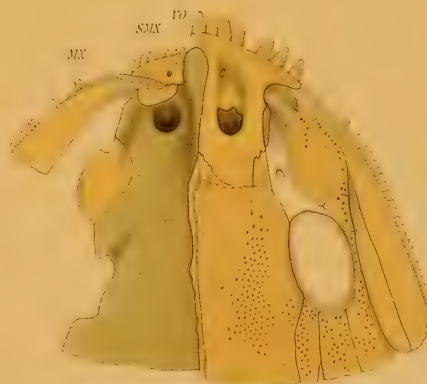
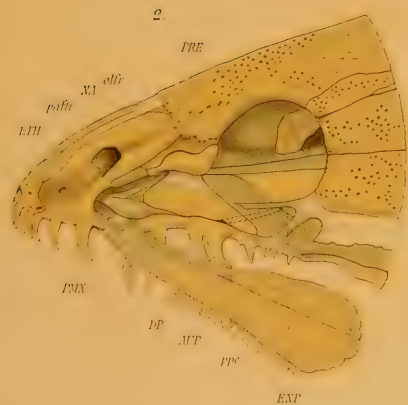
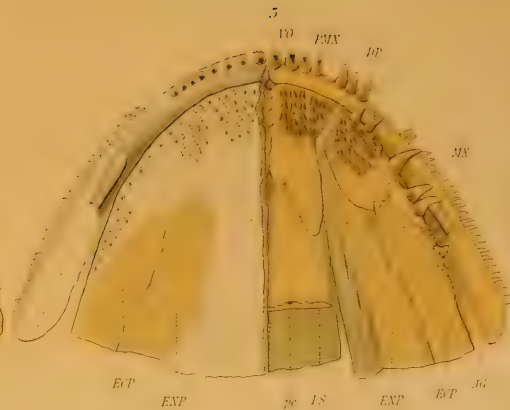
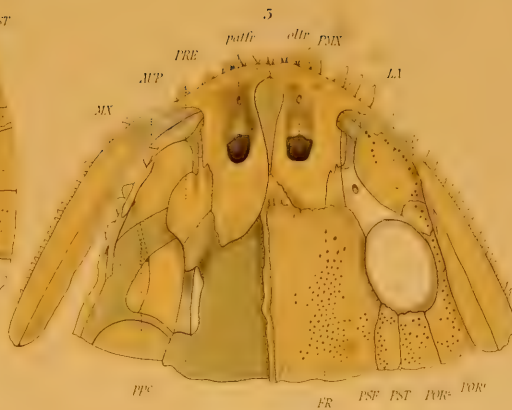
FIG. 5. Bottom view of same skull. The left side is undissected and shows the dorsal surface of the mouth cavity. On the right side the tissues covering the bones have been removed. The parasphenoid is cut somewhat shorter than the other bones.  $\times 2$ .

FIG. 6. Same as Fig. 5. On the left side the tissues covering the bones have been removed, as on the right side of Fig. 5. On the right side all of the dermal bones have been removed excepting the parasphenoid.  $\times 2$ .











## THE LOCATION OF THE BASIS OF THE AMPHIBIAN EMBRYO.

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THE query, "From what part of the amphibian egg does the embryo arise?" is but a natural outgrowth of the earlier researches of Vallisneri, Spallanzani, and Swammerdam. Although often answered by later investigators, — Prévost and Dumas, Rusconi, von Baer, Reichert, Cramer, Newport, and others, — their replies reveal a diversity of opinions.

The more recent researches of Pflüger, Roux, O. Schulze, O. Hertwig, Morgan and Tsuda, Assheton, and myself have likewise failed to settle the question, but have led to a more precise conception of the methods to be employed in attaining a solution. The problem might be restated as follows:

Is the darker (upper) hemisphere of the amphibian egg that portion which forms the basis of the embryo, as stated by Swammerdam, Prévost and Dumas, von Baer, Reichert, Cramer, Newport, and O. Schulze? Or,

Is the lighter (lower) hemisphere to be regarded as containing the basis of the embryo, as held by Pflüger, Roux, Hertwig, Morgan and Tsuda? Or,

Do considerable portions of both hemispheres contribute to the embryonic basis, as advocated by Assheton and myself?<sup>1</sup>

In an earlier paper the results of certain experiments on the egg of *Amblystoma* were recorded. These experiments seemed to clearly indicate that, at least, the anterior half of the embryo of *Amblystoma tigrinum* differentiates in an area lying between the upper pole and the point at which the blastopore first appears.

<sup>1</sup> The striking similarity of some of my conclusions, as given in the *Journ. of Morph.*, February, 1895, to those of Assheton, published in the *Quar. Journ. Micr. Sci.*, December, 1894, compel me to state that a copy of my paper was presented to the Biological Faculty of the University of Chicago on Dec. 15, 1894. The press work was completed when Assheton's paper was received, so that reference to the latter was precluded.



During the spring of 1895 similar experiments were made on the eggs of *Acris* and *Bufo*, and those made on the eggs of *Amblystoma* were repeated and extended.

Given areas were marked by the method used in the earlier work, which was briefly as follows: The outer envelopes are removed from the egg, which is then placed in a watch crystal, on a bed of cotton, with barely enough water to cover it. The egg is then rotated until the desired point is uppermost, when it is punctured with an extremely fine hair held by forceps. A small quantity of the protoplasm oozes out and forms a minute exovate, which remains attached to the egg. The eggs thus marked are transferred to watch crystals, in which they remain until the embryo is formed. A large mirror is fastened to the stage of a dissecting microscope; on this the watch crystals are arranged in series, and the eggs examined by means of an extension arm. The image of the opposite side may be thus observed during the entire period, from the time of marking until the embryo is formed.

The removal of the envelopes is necessary, since it is otherwise impossible to prevent the rotation of the egg, often resulting in the detachment of the exovate. If it be argued that the course of development is thus diverted, I reply by saying that I have compared the phases of cleavage and embryo formation in eggs from which the envelopes had been removed, with those in which the membranes were intact, and can positively assert that in these stages no visible disturbances are induced. In marking the eggs, great care is imperative, since a severe puncture invariably results in the formation of a large exovate, which necessarily obscures normal development; again, the puncture may be so slight that the injured portion heals, and the mark is entirely obliterated.

*Series I.—Acris grillus.*

On March 28, 1895, twenty-five eggs of *Acris* were selected, in which the second cleavage grooves were just forming. From these the envelopes were removed, and the eggs punctured at the point where the first and second cleavage grooves crossed. The eggs were then transferred to watch glasses, and examined

at frequent intervals. Eighteen eggs developed normally; in the remainder, the exovates were so large that either death or abnormal embryos resulted.

In eight eggs (Figs. 1, 2, 6-10, 13) the exovates were later found in or near the median plane of the embryo, and in the immediate vicinity of the transverse portion of the neural fold.

In three eggs (Figs. 14, 15, 17) the exovates lay within the transverse portion of the neural fold, and at the right of the median plane.

In two eggs (Figs. 11, 12) the exovates were found in the transverse portion of the neural fold, and at the left of the median plane.

In one egg (Fig. 18) the exovate was in the median plane, but far behind the transverse portion of the neural fold.

In four eggs (Figs. 3-5, 16) the exovates were entirely without the embryonic area. Those shown in Figs. 3 and 16 occupied positions which at present are quite unexplainable. It is possible, although improbable, that a shifting in the position of the exovates occurred. It should be here stated that the eggs were examined at short intervals, and the position of the exovates, with reference to certain accidental abrasions, carefully noted.

Other experiments were made by marking various portions of the egg in the vicinity of the blastopore.

Five eggs were selected in the crescentic blastopore stage. These were punctured just above the middle of the blastopore, as shown in Figs. 19 and 21. Three developed. In two the exovates gradually approached the blastopore, and were carried within the margin, as shown in Figs. 20 and 22. When they came in contact with the yolk mass they were detached. Their points of attachment, however, remained visible, and were followed step by step until they disappeared within the blastopore. In one egg the exovate passed through the successive steps represented by Figs. 28-30, but was not infolded.

Five eggs were marked at one extremity of the crescentic blastopore, as shown in Fig. 23. Only one egg developed. The position which the exovate finally occupied with reference to the blastopore is shown in Fig. 24.

Five eggs were selected in the crescentic blastopore stage, and punctured at a short distance below the centre of the blastopore, as shown in Figs. 25 and 31. Of these but two were satisfactory. The successive positions occupied by one of these are shown in Figs. 25-27, while those occupied by the other are shown in Figs. 31 and 32.

Three eggs in the stage of crescentic blastopore were punctured at points some  $15^{\circ}$  to  $20^{\circ}$  above the blastopore, as shown in Figs. 33 and 35. Two operations were successful. In one egg the exovate was later found within the caudal portion of the neural fold and near the median line, as shown in Fig. 34. In the other (Fig. 36) the exovate lay in the fold and at the left of the median line.

Four eggs in the crescentic blastopore stage were punctured below the middle of the blastopore, and in the immediate vicinity of the lower pole, as shown in Figs. 37 and 39. Two eggs developed normally. The exovate in one case occupied a position just below the posterior end of the embryo, as shown in Fig. 38. In the other (Fig. 40) it lay much farther below the posterior end of the embryo.

*Series II. — Bufo lentiginosus.*

May 5, 1895, five eggs in the early gastrula stage were punctured just below the equator and above the centre of the crescentic blastopore. The results are shown in Figs. 41-45. In all cases the exovates lay behind a transverse line marking off the posterior third of the length of the embryo. The variations existing in the position of the exovates, with reference to the median plane of the embryo, were probably due to the fact that the ends of the crescentic blastopore progressed at such varying rates that it was difficult to locate the meridian which passes through the centre of the blastopore.

In five eggs in late cleavage, punctures were made at the upper pole. Four of these developed. The positions of the exovates are shown in Figs. 46-49. In these cases they were in or near the transverse portion of the neural fold.

In ten eggs at the same stage, punctures were made in the same locality. In each of this lot a second puncture was made



just above the equatorial zone, and in a meridian passing through the centre of the crescentic blastopore. Seven eggs developed. In three (Figs. 50, 51, 54) the exovates were located in or very near the transverse portion of the neural fold. In one (Fig. 52) it was far without, and in one (Fig. 53) far within. In two eggs (Figs. 55, 56) the exovates at the upper pole were detached.

In those eggs in which the punctures were made at, or slightly above, the equatorial zone, the exovates occupied the positions shown in Figs. 50-56. In most cases they lay near a transverse line marking the middle of the embryo.

*Series III. — Amblystoma punctatum.*

The egg of *Amblystoma*, owing to its large size and the ease with which the membranes are removed, is well suited for experimental work. The results of the experiments performed are given in greater detail than in the preceding forms.

Ten eggs in second cleavage were selected at 8 A.M., April 1, 1895; three were punctured in the inner ends of two of the large cells lying on either side of the first cleavage groove, as shown in Fig. 59. In two of these the exovates were so large that abnormal embryos were formed. In one the exovates were very minute. At the end of seventy-two hours the neural fold appeared. The positions of the exovates, with reference to the neural fold and the median plane of the embryo, are shown in Fig. 60.

Three eggs were punctured at the inner ends of the large cells lying diagonally opposite, as shown in Fig. 61. In one the neural fold was visible at the end of seventy-three hours. The positions of the exovates, with reference to neural fold and the median plane, are shown in Fig. 62. In one case the exovates fused into a common mass, which, however, did not entirely obscure the normal relations. At the end of sixty to seventy-five hours, the position of the fused mass was far within the neural fold and at the left of the median line. The remaining egg died soon after puncturing.

Four eggs were punctured in the ends of the adjacent cells on the same side of the first groove, as shown in Fig. 63. In

two eggs the exovates were large. In two they were of the desired size. At the end of seventy to eighty hours the embryos appeared. In one egg the exovates occupied the positions shown in Fig. 64. In the other they were on the opposite side of the median line, and just outside of the neural fold.

At 9 A.M., April 2, five eggs were selected; of these, three were in the stage of crescentic blastopore, and two in stages a trifle later. These eggs were punctured in a region midway between the blastopore and the upper pole, as shown in Figs. 65, 67, 69, 71. In from twenty-five to twenty-six hours, four had developed normal embryos. The other died during gastrulation.

In two eggs the exovates were later found in the middle of the embryo and far to the left of the median line, as shown in Figs. 66 and 68. In one egg (Fig. 70) the exovate was in the middle of the embryo, but far to the right of the median line. In the other (Fig. 72) the exovate was in the median line, and at about the same level as in the preceding case.

At 8 A.M., April 3, an egg was selected, in which the early blastopore extended over an unusual distance; the egg was punctured just above the centre of the blastopore, as shown in Fig. 73. At 12 M. the blastopore had changed to the form shown in Fig. 74, and the exovate had progressed toward the lip of the blastopore. At 5 P.M. the circular form of the blastopore had been attained (Fig. 75). The exovate had now reached the verge of the blastopore; at 11.30 P.M. it was just at the anterior end of the linear blastopore. At 6 A.M. on the following morning, the neural fold had appeared, and the position of the exovate, with reference to this fold, is shown in Fig. 76.

At 8 A.M., April 3, an egg in the stage of early blastopore (Fig. 77) was punctured a short distance above the blastopore and directly over its middle point. At 5 P.M. the blastopore had reached the semicircular form shown in Fig. 78. The exovate had approached the blastoporic margin; its progress being much slower than in the preceding case. On the following morning the blastopore had reached the circular form shown in Fig. 79. The exovate had continued its progress toward the blastoporic margin. At 7 P.M. the neural



fold had formed, and the position of the exovate was found to be slightly at the right of the median line and in the posterior end of the embryo, as shown in Fig. 80.

At 3 P.M., April 4, an egg in which the blastopore had reached the crescentic form (Fig. 81) was punctured a short distance above the blastoporic opening. At 9 A.M. on the following morning the blastopore had narrowed to a small, circular opening, as shown in Fig. 82. At 6 P.M. the neural fold became visible, and the exovate was found to occupy a position considerably at the left of the median plane, and in the posterior end of the embryo (Fig. 83).

At 3 P.M., April 4, an egg in the stage of crescentic blastopore was selected, in which a group of peculiar accidental markings were present near the blastopore, as shown in Fig. 84. At 9 P.M. the blastopore had changed to the form shown in Fig. 85. It will be observed that the mark above the blastopore has passed toward the blastoporic margin at about the same rate observed in the progress of the exovates in the three preceding experiments. At 6 A.M. on the following day the blastopore had almost reached the circular form, as shown in Fig. 86. The mark above the blastopore has passed entirely within, while those below are about to disappear. In following the successive steps represented by Figs. 84-86, it is plain that a change has occurred in the relative positions of these markings; this shifting has undoubtedly been brought about through lateral pressure, which is now revealed by the greatly elongated and narrowly compressed cells.

At 3 P.M., April 4, an egg in the early blastopore stage was punctured at a point  $20^{\circ}$  to  $25^{\circ}$  below the centre of the crescentic line, as shown in Fig. 87. At 5 P.M. the blastopore had changed to the semicircular form indicated in Fig. 88. The exovate and dorsal lip of the blastopore are now appreciably nearer to each other. At 11 P.M. the blastopore had reached the stage shown in Fig. 89. The exovate, however, does not seem to have changed its relative position. At 6 A.M. on the following day the circular blastopore was completed (Fig. 90), the distance between the exovate and the dorsal lip being now greatly diminished. At 11 A.M. the circular blastopore was

much smaller (Fig. 91), and the distance between the dorsal lip and the exovate further reduced. At 2 P.M. the exovate disappeared entirely (Fig. 92).

At 5 P.M., April 4, an egg was selected on which a natural or accidental mark was found just without one of the extremities of the semicircular blastopore. Near this mark a slight puncture was made, as shown in Fig. 93. At 10 P.M. the blastopore had changed to the circular form represented in Fig. 94. The marks were at this time much nearer the blastoporic margin than in the earlier stage. At 6 A.M. on the following morning the blastopore had changed to the linear form. The exovate was not carried in, but lay just at the edge of the blastopore, as shown in Fig. 95.

After much searching, another egg was found with an accidental mark at one extremity of the crescentic blastopore, in the position shown in Fig. 96. Five to six hours later the blastopore had changed to the form shown in Fig. 97. At this time the mark had been carried to the very margin of the blastopore, as represented in the figure. Six hours later the blastopore had become circular in form (Fig. 98), and the mark had been carried into the blastopore.

### *Summary of Experiments.*

#### I.

Experiments 1, 2, 5-15, 17, 18 indicate that the upper pole in *Acris* represents that portion of the egg which later forms the anterior end of the embryo.

Experiments 46-54 show similar results in *Bufo*.

Experiments 59-64 show the same to be true of *Amblystoma*.

#### II.

Experiments 50-56 indicate that in *Bufo* an area lying midway between the upper pole and the point which marks the first beginning of the blastopore later forms the mid-dorsal portion of the embryo.

Experiments 65-72 show like results in *Amblystoma*.

## III.

Experiments 33-36 indicate that in *Acris* an area lying just above the point at which the blastopore first appears later forms a portion of the posterior end of the embryo.

Experiments 41-45 gave the same results in *Bufo*.

Experiments 73-83 show the same to be true of *Amblystoma*.

## IV.

(a) Experiments 19-22, 28-30 indicate that in *Acris* the cells immediately above the dorsal portion of the blastopore pass toward and into the blastopore.

Experiments 73-75, 77-79, 84-86 show the same results in *Amblystoma*.

(b) Experiments 23, 24 indicate that in *Acris* the cells on either side of the blastopore approach the same and are infolded.

Experiments 93-98 show the same to be true of *Amblystoma*.

(c) Experiments 25-27, 31, 32 indicate that in *Acris* there is an infolding of the larger cells forming the yolk plug, or that this mass is being overgrown by the smaller cells forming the blastoporic margin, or that both processes are combined.

Experiments 88-92 lead to the same conclusion regarding *Amblystoma*.

Experiments 84-86 show that an infolding of the smaller cells forming the blastoporic margin actually takes place, but is obscured to a great extent by the extension or overgrowth of the blastoporic margin over the yolk, which is going on at the same time.

*Some General Remarks.*

In order to obtain a clearer conception of the factors which determine the position of the embryo, it is necessary to recall that during the cleavage of the amphibian egg the cells at the upper pole divide most frequently, and give rise to an area of smaller cells, which, according to my earlier experiments, as well as those recorded under I, in the present paper, forms the basis of the cephalic end of the embryo.

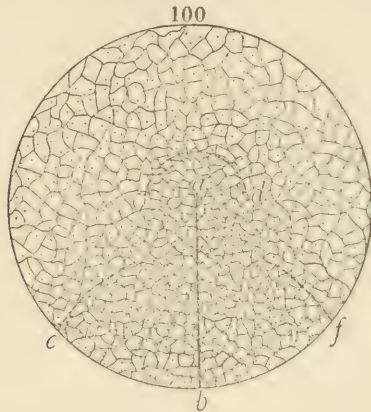
In those amphibian eggs which are deeply pigmented, an eccentric distribution of the pigment has been noted by a number of investigators. Professor Whitman, in lectures on vertebrate embryology, has repeatedly called attention to the unequal distribution of pigment in the amphibian egg, and emphasized its importance in the orientation of the embryo. Morgan and Tsuda (*Quar. Journ. Micr. Sci.*, 1894, p. 377) found an unequal distribution of pigment in the eggs of *Rana*, which they described as follows: "In all the eggs, from the earliest stages up to the blastopore, there is one marked peculiarity of the pigment. There is always a greater deposit of pigment on one side of the egg than on the other . . ." (p. 380). "The interesting point in connection with the two opposite, the darker and lighter sides of the eggs, on which I have dwelt at such length, is that the less densely pigmented half of the egg very early in the segmentation shows signs of a more rapid development and growth than the darker and pigmented side . . ." (p. 381). "The blastopore makes its first appearance on the less pigmented and further developed side of the egg." My observations on the eggs of *Amblystoma* (*Journ. of Morph.*, 1895, p. 367) led to the following conclusion: "In all the eggs where the differences are sufficiently marked to admit of orientation, the irregular line (blastopore) lies beneath the darker portion and is parallel with its more sharply defined border. It will be recalled that the deeply pigmented area is the area in which cell division has become much accelerated."

It is obvious that the conclusions of Morgan and Tsuda and my own, concerning the distribution of pigment, are at variance. As to the position of the secondary area of cell activity, however, there is complete agreement. Assheton has also described this area. He states that: "Possibly the secondary area of proliferation may start immediately upon the formation of the dorsal lip of the blastopore, and not delay until the whole blastoporic rim is completed." While there are minor differences of opinion as to the extent and time of origin of this area, there is no question as to its location or significance. The experiments recorded under III seem to show that this area gives rise to the greater portion of the posterior half of the embryo.



It is of interest to note that the location of this area of increased activity corresponds in position to a like area described by Lwoff in *Amphioxus*, Kölliker, Kionka, and others in the chick, and Assheton in the rabbit.

In order to give a more graphic conception, I have introduced the accompanying diagram (Fig. 100). It represents the upper



hemisphere of an egg of *Amblystoma* in late cleavage. The dots represent pigment, which on one side of the upper hemisphere is much denser than on the other. This portion of the egg which is most deeply pigmented is likewise the portion in which cell activity is greatest. This activity is due to two distinct areas of accelerated growth. The primary lies at the upper pole within the broken semicircular line *c-d*. The secondary is less easily defined. It may be considered, however, as lying within the arcs *e* and *f*. These two areas define the position of the embryonic tract, and a line *a-b* drawn through their centres represents the antero-posterior axis of the future embryo.

The formation of the posterior portion of the embryo is due to a number of imperfectly comprehended forces, and it is consequently obvious that any explanation will be little more than a working hypothesis.

There is no question but what the cells lying just above the dorsal lip of the blastopore are especially active. This necessitates either an infolding or a backward extension of the



embryo, or both. The facts recorded under IV show that an infolding occurs, while those given under III leave no doubt as to the backward extension of the embryo.

When the blastopore has reached the circular form, there is an infolding around its entire margin and a consequent extension of the secondary area toward the ventral lip, so that the process of infolding varies at different points in the margin of the blastopore, the point of least activity being at the ventral lip.

Soon after the completion of the blastopore two areas of rapid growth are apparent on either side of the circular blastopore. These are the *Anlagen* of the neural folds. A new factor is here introduced, which may explain how the blastopore changes from the circular to the linear form.

The facts presented in the preceding pages lead to the following conclusions:

The primary area of cell activity, at the upper pole of the amphibian egg, forms the basis of the cephalic end of the embryo.

The secondary area of cell activity, on the blastoporic side of the egg, forms the basis of the greater portion of the posterior half of the embryo.

These two areas constitute an embryonic tract, from which arise at least the anterior two-thirds of the embryo.

The posterior end of the embryo is formed by a coalescence of the lateral portions of the blastoporic margin.

*The greater portion of the embryo arises in the darker hemisphere by differentiation in situ, and not by concrescence.*

HULL ANATOMICAL LABORATORY,  
UNIVERSITY OF CHICAGO,  
March 20, 1897.



## EXPLANATION OF FIGURES.

All figures drawn from living eggs  $\times$  about 15.

FIGS. 1-18 represent eggs of *Acris*, in stage of neural fold, viewed from the anterior end.

FIGS. 19-40 represent eggs of *Acris*, viewed from the posterior (blastoporic) end.

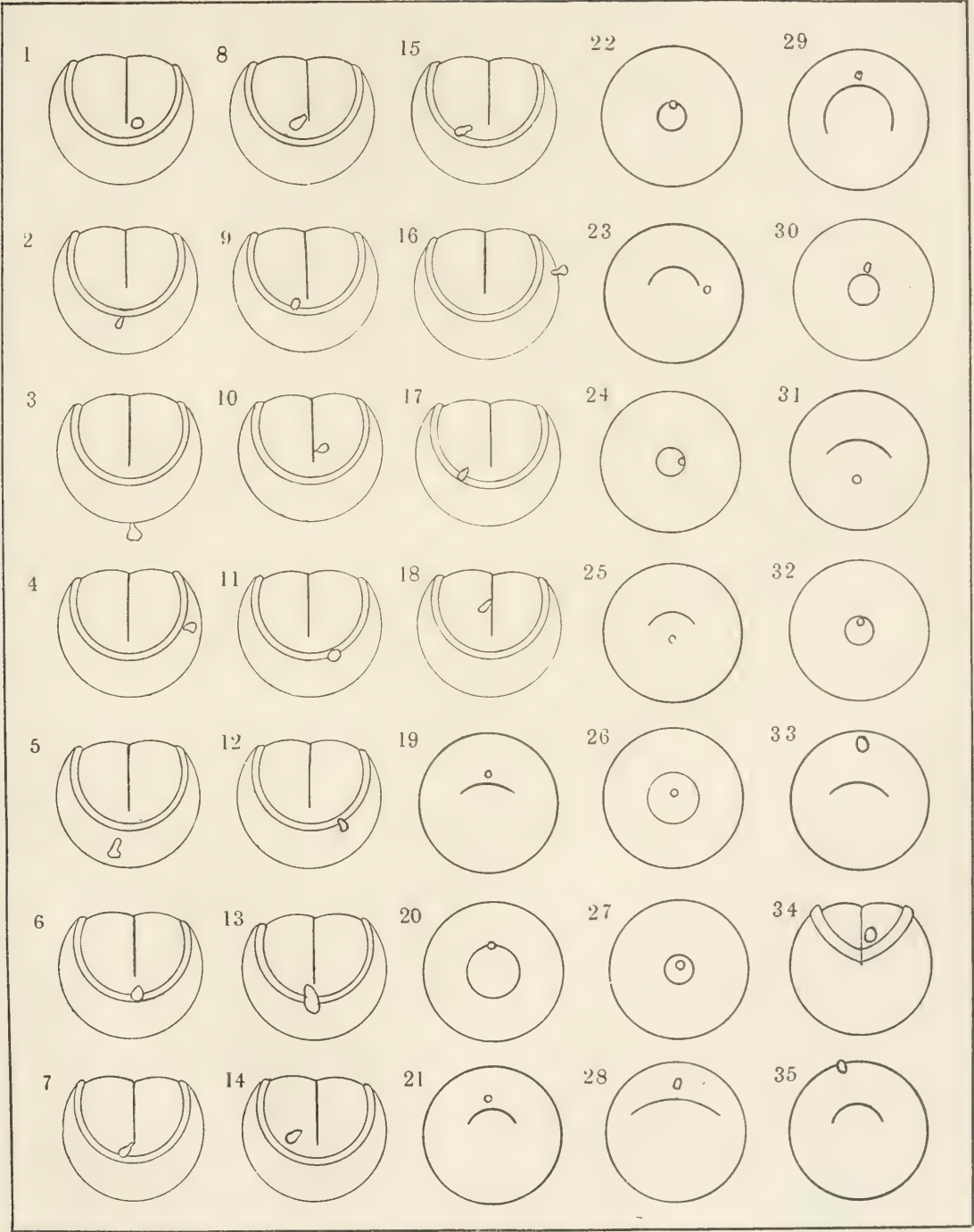
FIGS. 41-56<sup>1</sup> represent eggs of *Bufo*, in stage of early neural fold, dorsal aspect.

FIGS. 59, 61, 63 represent eggs of *Amblystoma*, in stage of second cleavage, viewed from upper pole.

FIGS. 60, 62, 64, 66, 68, 70, 72, 76, 80, 83 represent eggs of *Amblystoma*, in stage of early neural fold, dorsal aspect.

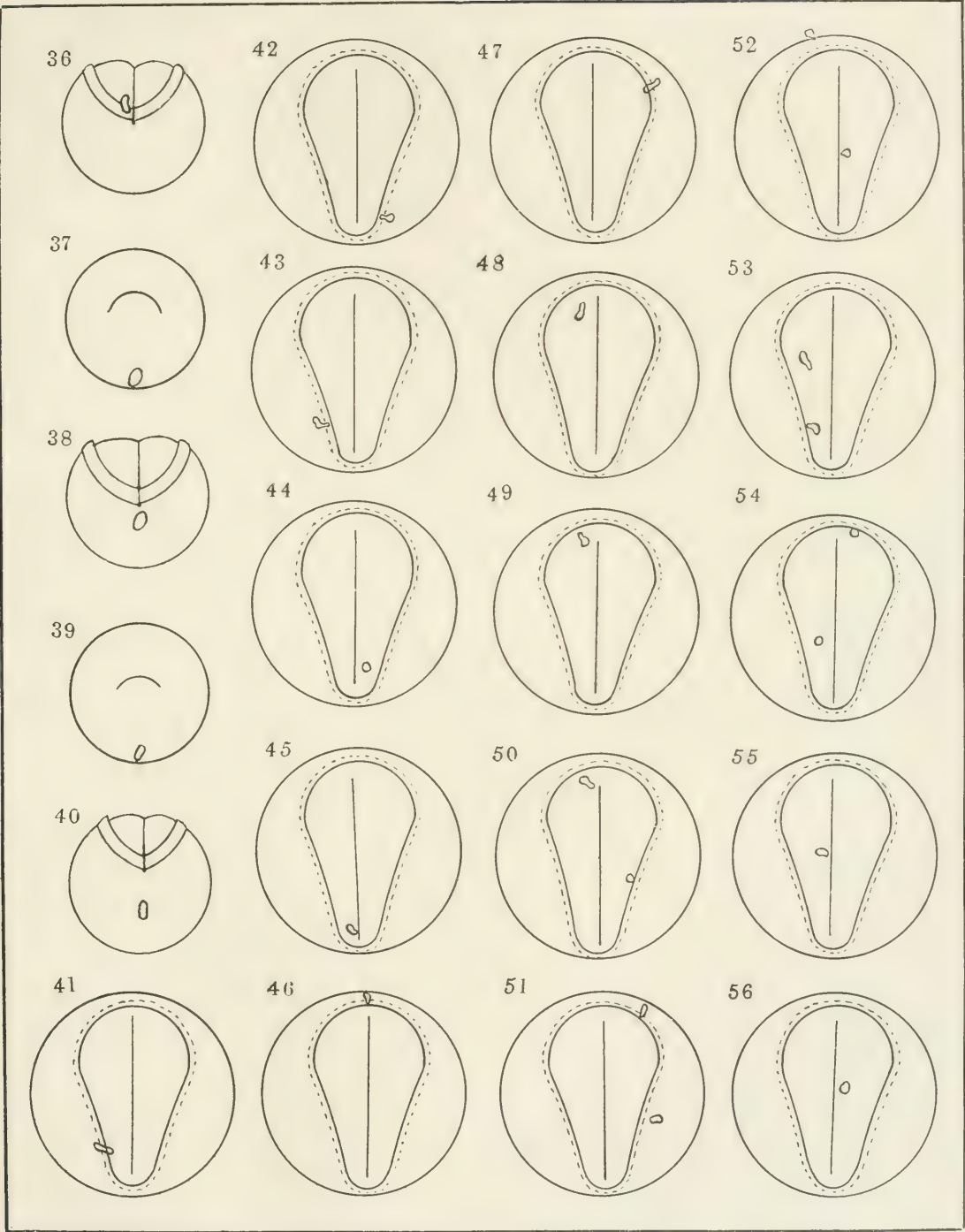
FIGS. 65, 67, 69, 71, 73-75, 77-79, 81, 82, 84-98 represent eggs of *Amblystoma*, viewed from posterior (blastoporic) end.

<sup>1</sup> Owing to necessary changes in arrangement of figures, Nos. 57, 58 were omitted.

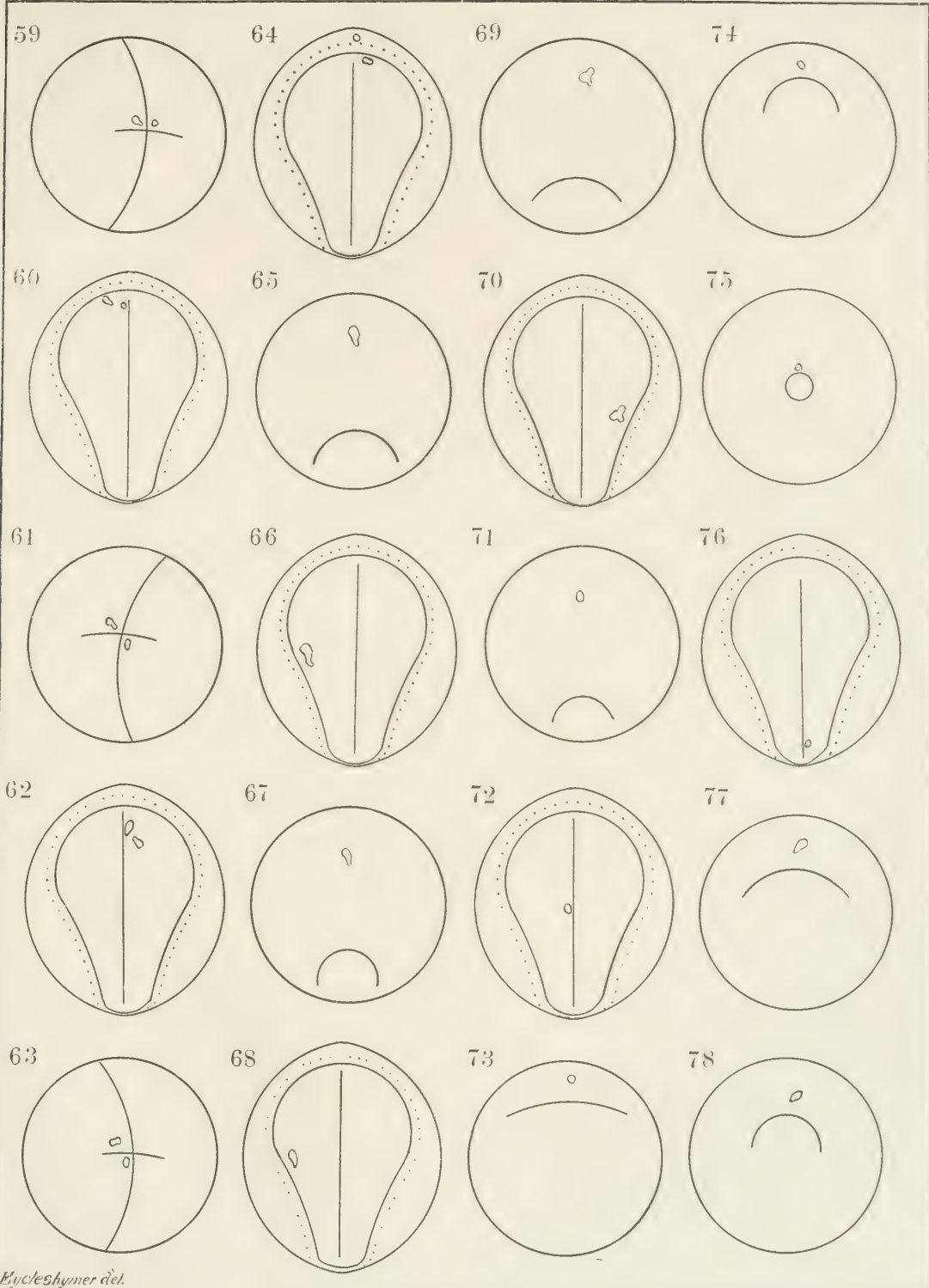




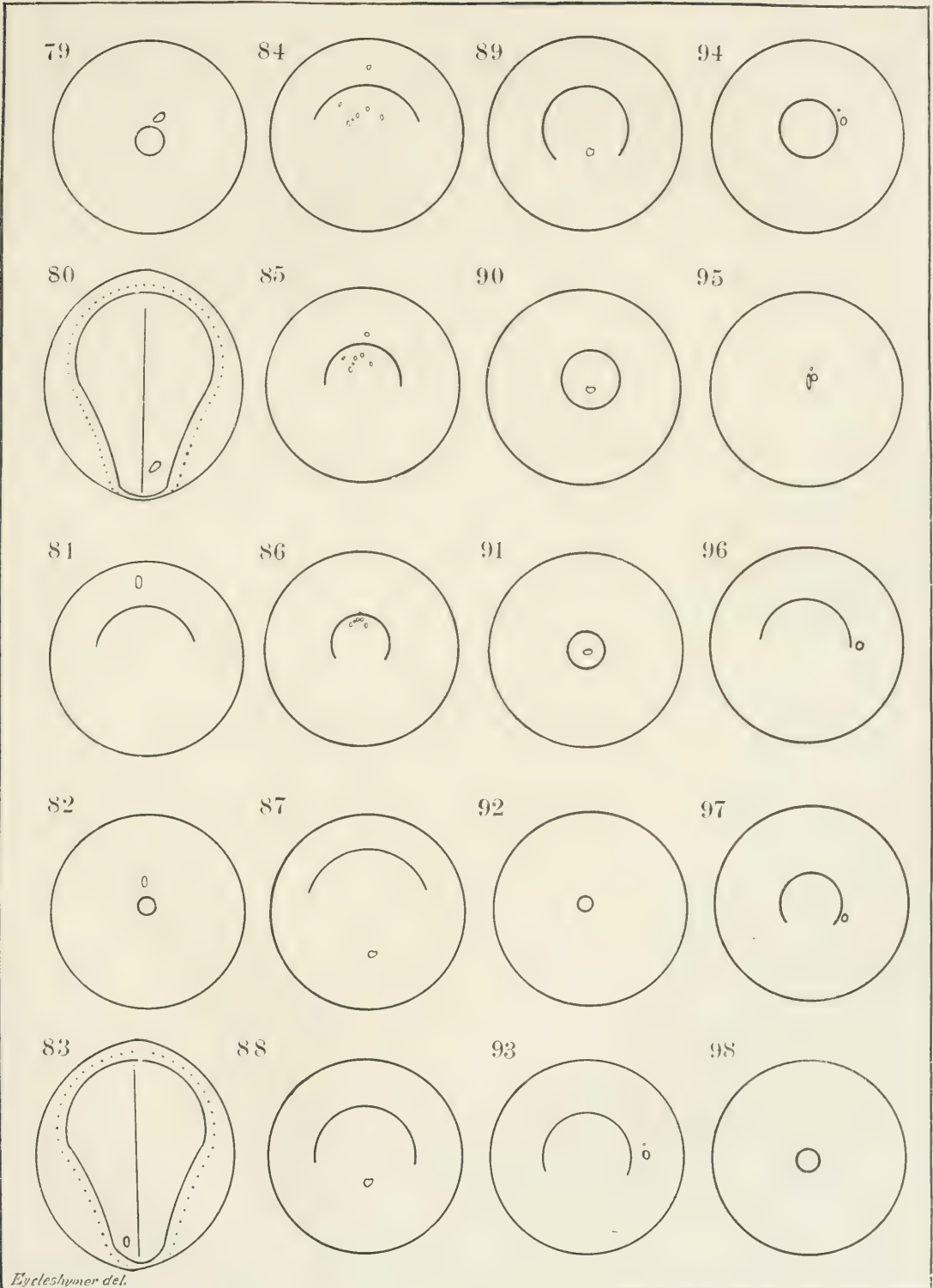
















## THE COCOONS AND EGGS OF ALLOLOBOPHORA FOETIDA.

KATHARINE FOOT.

INVESTIGATORS (1) (3) (11) (20) who have observed the copulation of earthworms mention a slimy substance secreted by the two worms during union, which encircles the pair around those segments that are in contact. In March, 1893, while examining a pair of worms surrounded by this slime, its form led me to suspect that two cocoons are formed during copulation. Within a few days this surmise was strengthened by finding a freshly deposited cocoon (Fig. 3) encased in a slime-covering entirely similar to that which surrounds the copulating worms. In the following summer I was able to witness the deposition of the cocoons and to find many worms with cocoons in the early stages of formation.

Fig. 1 represents about fifty of the anterior segments of two copulating worms. The anatomical features have been so frequently described that it is necessary here only to call attention to the covering of transparent slime that completely surrounds the two worms from the 8th to the 33d segments. In the figure I have represented the average limits of this substance. These limits vary in the 200 or more worms examined ; but I am inclined to think the variation is due largely to the efforts of the worms to escape during the process of capturing and killing them. They were quickly seized and dropped into boiling water, the entire process occupying only one or two seconds, but that would be sufficient time to allow the worms to change the position of several segments in relation to the slime surrounding them.

Fig. 2 represents this slime-covering after the worms have been disturbed and allowed to withdraw from it. If placed in water, it will regain its original shape and will be found to be tube-like, sometimes showing the impress of the seg-

ments of the worms, and so elastic that after pressure it springs back to its original shape. At this stage, and when encasing the cocoon (Figs. 3 and 4), its form is so tube-like that I shall designate it the slime-tube. It can be injected from both ends; in some cases the colored fluid will flow freely through the entire tube, and again it will fill only one-half, and the other half can be injected with another color.

I am inclined to think this semi-independence of the two tubes is the usual condition; for after deposition each cocoon is encased by its own tube (Figs. 3 and 4). There is, however, an intimate connection between the two; for when the copulating worms are disturbed, in many cases one worm will escape first, leaving both tubes around the other worm. When the slime-tube is torn, as seen in Fig. 2, the torn portion can be peeled off from the rest as though it were a separate layer. The entire layer can be pulled off backwards with a pair of forceps, — can be turned inside out without tearing.

Within the slime-tube flows the seminal fluid, containing free spermatozoa and spermatophores. I designate as spermatophores the branched bundles of spermatozoa which are aggregated at the distal ends of the slime-tube (text Fig. 2). During the early stages of the formation of the cocoon, these completely cover the dorsal surface and sides of segments 9 to 11 of each worm and are packed between the two worms at this region. The contents of a partly formed cocoon, when shaken in water, break up into several of these formations, often as many as six, many of them as large as that represented in text Fig. 2. I have never found any eggs in the slime-tube before the cocoons were partly formed, this fact indicating that the eggs are deposited towards the end of copulation. As these worms copulate at a greater or less distance beneath the surface of the earth, at least one function of the slime-tube must be to protect and confine the seminal fluid and spermatophores. If this interpretation be correct, we should find this slime-tube encasing all freshly deposited cocoons of worms which copulate beneath the surface of the earth. I have found this to be the case for several species, and

Vejdovský (19)<sup>1</sup> has figured for *Lumbricus rubellus* a structure which unquestionably answers to the slime-tube of *Allolobophora foetida*, though he does not suggest any connection between this "schleimartiger Fortsatz" and the copulating worms. He states also that in all the species of the Lumbricidae studied by him, he finds a like structure in connection with the freshly deposited cocoons.

*Both cocoons are formed while the worms are united*, and when they separate each deposits a cocoon, encased by a moiety of the slime-tube. During five summers devoted to collecting and preserving this material, I have seen many cocoons deposited, and in some cases have found the worms copulating so near the surface that by carefully removing a little of the earth, I could watch them for hours, and finally secure and open both cocoons. The examination, however, has necessarily been superficial; for if enough earth is removed to allow an examination with a lens, the worms soon become restless and slowly move away below the surface. In one case they remained undisturbed from 9 A. M. until 2.52 P. M., and I was then able to secure one of the cocoons, though I was not quick enough to catch the second worm. The worms separate quickly, each drawing back from the other into the hole in which the posterior part of its body has remained during copulation. In some cases they leave the two cocoons attached to each other by the slime-tube; but I am convinced that this is not the usual process, but is due to the worms being disturbed; for in those few cases where I was able to watch without disturbing them, only one cocoon was deposited at the spot where the worms had copulated, the second being deposited at some distance from the first. Each cocoon appears to be formed around both worms, encircling the clitellum of one worm and three or more of the anterior segments of the other worm (Fig. 1). As the worms withdraw backwards out of the cocoons, the eight free anterior segments of each must be withdrawn first, leaving a cocoon around the clitellum of each worm; the worm finally leaving this cocoon at the end

<sup>1</sup> His figure differs from my Fig. 3 mainly in the fact that the ends of his cocoon are reversed in relation to the slime-tube.

represented in Fig. 3 by the thread-like extension. Owing to the rapidity with which the worms separate, I have not been able to satisfy myself definitely on several points relating to the formation and deposition of the cocoons.

In Fig. 1 are seen four denser, cord-like portions of the slime-tube, circling the anterior and posterior edges of the clitellum of each worm, and binding closely against it three (9-11) of the anterior segments of the other worm. These cord-like portions seem to have contracted until they press into the bodies of the worms like a thread tightly wound around them, and they are so tough that by slipping a needle under any one of them, both worms can be lifted from the table in spite of their efforts to separate and escape. Possibly they are differentiations of the slime-tube; possibly they are of the same secretion that forms the cocoon, though they are present before the *slightest indication* of a cocoon can be detected. They narrow the lumen of the slime-tube (Fig. 2) in planes finally occupied by the ends of the cocoons, suggesting that they may later aid in closing the cocoons.

When the cocoon is first deposited, its case is *perfectly white*, less opaque than the albumen within it, and nearly as soft as the slime-tube. It does not acquire the slightest tinge of yellow until some minutes after deposition. Exposed to the air, it very rapidly changes color, becoming first a delicate pinkish straw-color and assuming later the more distinctly yellow tone. As it acquires this yellow tinge, it becomes more resistant, and finally attains its hard chitinous character. An immature cocoon changes color much less rapidly. After opening such a one and preserving the eggs, the case of the cocoon remained white for more than an hour, and after five hours had acquired only a delicate yellow tinge. This appears to indicate that the secretion which forms finally the chitinous constituent of the case is not formed at the earlier stages of the construction of the cocoon.

The slime-tube of the freshly deposited cocoon is transparent, adhesive, and elastic, adhering so closely to the soft white cocoon that it seems part of it and it is difficult to separate the two. As the cocoon becomes yellow and attains its hard



chitinous consistency, the slime-tube loses its slime-like character and elasticity, can be easily torn and separated from the cocoon, and finally entirely disintegrates. Fig. 4 shows a slime-tube around a cocoon, probably containing eggs in the pronuclear stages. The tube was found torn away from one end of the cocoon, and its tube-like character is shown by the fact that parts are telescoped into each other. Sometimes the tubes can be turned completely inside out, like the finger of a glove. The time required for the disappearance of the slime-tube depends largely upon the character of the earth in which it is deposited. I have been able to preserve it many hours, and again it will disintegrate in two or three hours. Sometimes a remnant will be found adhering to a cocoon containing eggs in the second and third cleavage stages; but as a rule, there is only a part of the slime-tube left around those cocoons containing eggs in the first cleavage stages.

The persistence of the slime-tube until the cocoon has acquired its chitinous character suggests that it may have a protective value for the freshly deposited cocoon.

As a rule, in cocoons opened just after deposition, the maturation spindle is at the metaphase and either at the center of the egg or at the periphery (Fig. 5). In these cocoons only an occasional egg shows a spermatozoön just penetrating the periphery. Of the many cocoons either seen deposited or found while still white and soft, I have preserved the eggs from thirty-one, and only two out of this number are exceptions to the above rule. In one of these exceptional cases all the eggs of the cocoon contained a first cleavage spindle.<sup>1</sup> In the other, one of two freshly deposited cocoons found side by side contained oöcytes of the second order. This was not due to delay in opening the cocoon, for these eggs were found in the cocoon first opened, while the second contained the usual oöcytes of the first order (Fig. 5). I have preserved many freshly deposited cocoons with a view to testing the rate of development, but this uncertainty as to

<sup>1</sup> It is impossible to confound the first maturation spindle with the first cleavage spindle; for in normal eggs the latter is always accompanied by pronounced polar rings.

the stage reached by the eggs in fresh cocoons makes these experiments of little value. After preserving a fresh cocoon for  $1\frac{1}{2}$  hours in the compost at a temperature of 21 C., the slime-tube was still comparatively fresh and the cocoon contained oöcytes, second order, with sperm attraction-sphere and rod.<sup>1</sup>

Another cocoon, similarly preserved for two hours, contained eggs showing exactly the same stage of development. The eggs in another cocoon, similarly preserved for three hours, had reached the pronuclear stages. In cocoons opened about ten minutes after deposition, only an occasional egg has remained unfertilized, the rest showing the head of the spermatozoa just penetrating the egg, or having passed its periphery.<sup>2</sup>

The total number of normal eggs<sup>3</sup> in 100 cocoons was 399, or about four to a cocoon, which may serve for a rough estimate of the number of normal eggs in each cocoon. At the height of the breeding season, however, the average of normal eggs is greater; for towards the end of the breeding season (after September 1) a cocoon is often found to contain only one normal egg. In the above-mentioned thirty-one freshly deposited cocoons, the average of normal eggs is about the same; thus the causes which produce the many structurally disintegrated eggs found in each cocoon must be sought in conditions prior to the deposition of the cocoons. I have opened 453 cocoons and preserved about 1900 eggs, in varying stages of development prior to the first cleavage. It appears to be the rule that the normal eggs in each of these cocoons have reached very nearly, if not quite, the same stage of development. For example, in a cocoon containing 19 normal eggs, 18 are oöcytes, 2d order, the spindle having reached the telophase (*i.e.*, the chromosomes appearing as small vesicles) while the sperm rod has reached the same stage. Only one of these nineteen eggs is an oöcyte, 2d order, with the spindle still at the metaphase.

Again, among 228 eggs taken from 50 cocoons containing

<sup>1</sup> For figures of egg at this stage, see Foot (5), Fig. 3; (6), Fig. 10.

<sup>2</sup> For figures representing the last stage, see Foot (5), Fig. 1; (6), Fig. 9; (8), Fig. 2.

<sup>3</sup> I use the term "normal" here, merely to designate those eggs that do not show any marked disintegration of the cytoplasmic or other structures.

eggs in the pronuclear stages, I find only eight eggs with a cleavage spindle and one with the first cleavage completed. The most marked cases of unequally developed eggs in the same cocoon are those in which one or more of the eggs has reached the metaphase of the first cleavage spindle, while the rest are oöcytes, 2d order, — the sperm being still at the rod stage.<sup>1</sup>

I have found only two such cocoons, and in one of these the retarded eggs show varying stages of structural disintegration.

Vejdovský, in his classic work, "Entwicklungsgeschichtliche Untersuchungen" (19), describes the freshly deposited cocoons of the Lumbricidae as "ziemlich weich," whereas the cocoons of *Rhynchelmis*, which he has *seen* deposited, he designates as "ganz weich" (p. 36). This, added to the fact that among his figures of eggs of *Allolobophora foetida* are none showing a first maturation spindle or a fertilization cone, convinces me that the youngest eggs of *Allolobophora foetida* found by Vejdovský are those represented in his Figs. 8 and 9, Pl. XIII, *viz.*, oöcytes, 2d order (fertilized eggs, with the first polar body formed, metaphase of second maturation spindle, and male attraction-sphere), though he has omitted the sperm rod. His figures of *Lumbricus rubellus*, however, show somewhat earlier stages (Taf. XIII, Figs. 1-4). In Figs. 2-4 he represents a structure undoubtedly answering to the cones of *Allolobophora foetida*, though he saw no sperm thread in connection with it, and his description (p. 68) of the probable method of fertilization is not supported by the facts, as seen in *Allolobophora foetida*. In Pl. XIII, Fig. 10, Vejdovský has figured what he interprets as an unripe egg; but I am compelled to question this interpretation and for the following reasons: only in the ovaries have I found unripe eggs, — eggs in the germinal vesicle stage, — never in the cocoons, not even in those *seen* deposited. This forces me to doubt the possibility of such eggs being found in cocoons containing eggs in the cleavage stages.<sup>2</sup> As stated above,

<sup>1</sup> Foot (6), Fig. 10.

<sup>2</sup> Vejdovský (19), p. 40: "Dass das Ei der weiteren Entwicklung nicht fähig ist, beweist der Umstand, dass ich es stundenlang ohne jede Veränderung beobachtete, während die übrigen Eier desselben Cocons in der Furchung begriffen waren."

greatly retarded eggs disintegrate beyond any structural recognition. Thus each cocoon contains eggs at about the same stage of development.

I am convinced that Vejdovský's Fig. 10 represents an unfertilized egg with the female pronucleus formed. I have found many such in relatively fresh cocoons, *i.e.*, those retaining part or all of a disintegrating slime-tube. Sometimes only one such egg will be contained in a cocoon with several others having one or more male pronuclei, and again a larger proportion of unfertilized eggs will be found, while in a few cases not even one egg will have been fertilized. The fertilized and normal eggs *always* show most pronounced polar rings,<sup>1</sup> whereas in the unfertilized eggs the polar rings often show various abnormal conditions. In some eggs only one is formed; in others the polar ring substance is still confined to the periphery of the egg (not having aggregated at either pole); in others, again, it is scattered throughout the cytoplasm. The only other figure of which I am aware that represents an unsegmented egg taken from the cocoon, is that by E. B. Wilson (21), Fig. 1. As, however, he shows neither polar rings nor pronuclei, the figure undoubtedly represents a disintegrating egg. The fixative recommended by Wilson himself (Perenyi's), if applied to normal eggs, has never failed to show pronuclei and polar rings. The latter structures are so pronounced that they can be seen in the living egg under a dissecting microscope.

*Breeding Season.*—The breeding season may be said to begin with the warm days of spring and to close when the nights become cold in the fall. Thus, of the five years I have devoted to collecting material in Evanston and Woods Holl, no two have begun or closed at exactly the same time. As the breeding worm shows a most marked clitellar region, it is a very simple matter to decide when the season has closed. Worms that, a few days before, showed pronounced clitella, will have that region only faintly marked, or quite indistinguishable from the rest of the segments. Experience has taught me that when a large proportion of worms in a compost heap show these indications of having stopped breeding, it is a waste of time to

<sup>1</sup> For figure representing this stage, see Foot (5), Fig. 7.



collect the material, — *i.e.*, to select from these worms those that still possess a marked clitellum. They may continue to deposit cocoons for some days, but as a rule the eggs in these cocoons are either entirely disintegrated structurally, or show abnormal features. At Woods Holl, the close of the season has varied from September 1 to October 1. It has been possible to collect worms with the clitellar region still marked, and to find fresh cocoons as late as October 20; but few of those cocoons have contained normal eggs. The breeding season closes much earlier in very old compost heaps, — those receiving no warmth from fresh manure. As early as August 15, in such a compost heap containing thousands of worms, it required a search of three hours to find fifty breeding worms. I can support Wilson's statement (21) that "egg-laying seems in special cases to continue throughout the year . . . but only in decomposing compost heaps, where the temperature is maintained at a tolerably high point" (p. 394). In 1893-94 I found breeding worms and cocoons in December, January, February, and March, but only in compost heaps that were covered with fresh manure.

*Method of Obtaining the Fresh Cocoons.*<sup>1</sup>— I select from a compost heap about a hundred full-grown breeding worms, *i.e.*, those having the clitellar region most pronounced, and place them in a one-gallon earthen pot, filled with the compost in which they have been found. To prevent the worms from escaping, it is well to tie over each pot a cloth, with a hole in the center.

In order to maintain the average of normal eggs in the cocoons, it is advisable to change the compost about once a week, and to collect a new supply of worms every two or three weeks. If the compost is kept dry, the worms copulate and deposit their cocoons near the bottom of the pot; but if it is kept moist, they come very near the surface.<sup>2</sup> I have fed them with various vegetables and fruits, but have found that the best method is to renew the compost, and to maintain the proper

<sup>1</sup> I use the term "fresh cocoons" to designate those still surrounded by the slime-tube and containing eggs no further developed than the first cleavage spindle.

<sup>2</sup> These facts support Vejdovský's observations on this point (19), p. 37.



degree of moisture and temperature. It has proved best to keep them as nearly as possible in a temperature of 21 C., avoiding extremes of heat or cold. Cocoons are deposited at all hours of the day, as fresh ones are found at all hours, varying from 4 A. M. to 6 P. M. They are undoubtedly deposited also at night, as I have found cocoons containing 2-, 3-, 4-, and 6-celled stages at 5 A. M. In midsummer, the early morning hours have proved most favorable for finding the fresh cocoons; but when the nights are cold, better success has been obtained later in the day. At the height of the breeding season from 2 to 10 fresh cocoons have been found daily in a pot containing 100 worms, and the number of cocoons found in these pots leads me to surmise that the worms deposit cocoons very often.

*Technique.*—The cocoon is placed in a small watch-glass under distilled water, and separated from its slime-tube by grasping the long end of the slime-tube with a pair of small, toothed forceps, and with a fine needle tearing it from the cocoon sufficiently to allow the latter to be pushed out. The cord-like projection of the cocoon is then held by the forceps, and with a sharp pair of scissors about one-sixth of the cocoon is cut off at its opposite (blunt) end, the albumen with the eggs being then readily pushed out by pressing the cocoon with the needle. The amount of the cocoon that can safely be cut off is easily determined, for the position of the eggs is readily observed under the dissecting microscope. When the cocoon is first deposited, it is difficult to separate it from its slime-tube, for the former is then so soft that the two appear almost fused. At this stage it is sometimes advisable not to attempt to separate them, but to cut through both with sharp scissors, removing the albumen by pressure. The eggs are set free from the albumen by teasing the latter with very fine needles. The albumen of the freshly deposited cocoons is so adhesive and elastic that it is very difficult to separate it from the eggs without injury to the latter. It gradually loses these properties, however, until by the time the cocoon contains oöcytes, 2d order, the albumen can be readily cut or torn by the needles, allowing the eggs easily to be set free. To avoid losing the

eggs, it is necessary to draw only one egg at a time into the pipette, and for this purpose it is advisable to make extremely small pipettes. Those that have proved the most serviceable have a final aperture of two-thirds of a mm. and are 6 cm. in length. The advantage of the latter is that it allows the hand to rest on the stage of the dissecting microscope.

The fixative recommended by Vejdovský (19), chromo-acetic, has proved the best for whole mounts, and allows the entire egg to be studied under a very high magnification. Alum cochineal has proved the most satisfactory stain for these whole mounts.

In order to study these eggs with a Zeiss 2 mm. immer., it is necessary to mount them with great care, as this lens has a working distance of about .2 mm. and many of these eggs measure .15 mm. I found it necessary to make glass feet by cutting square cover glasses of the proper thickness into narrow strips, about 15 mm.  $\times$  3 mm. It is safer to use four of these, inserting half their length under the cover, in order to be able to push it in any direction a sufficient distance completely to revolve the eggs. It is essential to ascertain the diameter of the egg, the thickness of each foot, and the thickness of the cover glass. A working distance of .23 mm. can safely be allowed, as it is not essential to focus entirely through an egg. If a first cleavage stage measures .16 mm. one can safely use feet measuring .17 mm. and a cover .06 mm. These very thin covers can be obtained only by special order. The feet are now in the market ; but as their thickness is variable, it is not safe to use them without remeasurement. They can, however, be cut in the laboratory as quickly as those of cardboard. This method is given in detail to avert any blunder similar to one made by myself. In the fall of 1893 I was obliged to remount all the material collected the previous summer, as the mounts were made with the regulation paper feet. It was only after much experimenting with both feet and covers, that the above method was reached. Thin layers of isinglass were first utilized as covers, but they have too much spring to admit of being safely pushed over the feet, and are thus apt to crush the eggs. Any number of eggs can be

safely oriented on the slide by using a single hair inserted in the regulation needle holder.

The general distribution of this material and the possibility of studying the whole egg under a high magnification make it of especial value for class work, and I publish the above detailed description of obtaining and mounting the whole eggs, in response to requests from those who wish to use them for demonstration in the class room. For the present, however, I reserve the publication of my methods of imbedding and sectioning.

As only a few hundred eggs can be collected each year, the time required for obtaining all the successive steps in the development is necessarily prolonged and uncertain, and I shall fill later such gaps as I am now obliged to leave. In the present paper I shall briefly state a few of my earlier observations.

#### OBSERVATION ON THE LIVING EGG.

*(Zeiss ob. 16 to hom. immer. 2 mm. 140 ap.)*

The egg must be removed from the albumen in order to study it with high powers. Distilled water (a few drops), with as much of the albumen dissolved in it as is consistent with preserving the necessary degree of transparency, has proved the least injurious medium for examination. In this the eggs have grown and developed normally from 30 to 60 minutes. The normal condition has been tested by removing the eggs from this medium, at definite periods of time, killing, imbedding, and sectioning them for comparative study. The following phenomena have been seen in the living egg: the maturation spindle in the unfertilized egg, and an indication of the rays of its attraction-sphere; the fertilization of the egg, *i.e.*, the appearance of the cone; the constricting off of the first polar-body, and the subsequent disappearance of the cone; the constricting off of the second polar-body, and the appearance of the polar rings. Not all these phenomena, however, are seen in any one egg; for the artificial conditions in which the egg must be studied soon arrest normal development. The first

appearance of the cone is indicated by a lighter area at the periphery, this projecting beyond the periphery and the projection continuing for some minutes after the cone is complete. The cone remains sharply differentiated from the rest of the cytoplasm during the constricting off of the first polar-body. The spindle is indicated by a lighter area clearly differentiated from the rest of the egg. The time occupied by the formation of the first polar-body has varied from 22 to 45 minutes, and I am inclined to think that the artificial conditions hasten development; for in the above-mentioned cases (p. 484) where fresh cocoons were kept in the compost  $1\frac{1}{2}$  and 2 hours, the eggs were still oöcytes, 2d order. The changes in the shape of the egg during the process agree with those described by Vejdovský (19), p. 51, Fig. 2) for *Rhynchelmis*, except that as the constricting progresses, the periphery of the egg at that point flattens, and finally becomes concave, the polar-body resting in the center of the concavity. At this stage, the membrane over the polar-body is so taut that the latter is often somewhat flattened by its pressure, suggesting that this pressure of the polar-body against the periphery of the egg may be at least partly responsible for the above-mentioned concavity. The phenomenon as seen in the living egg accords entirely with my observations on fixed material. A small part of the cytoplasm appears to be pushed out by the spindle, the latter first moving to the periphery and then projecting beyond it until its equator has reached the periphery of the egg, when the process of constriction takes place. The time occupied by the formation of the second polar-body has varied from 20 to 60 minutes, the two phenomena (the formation of the first and second polar-bodies) being similar. In each case a lighter area first appears at the periphery, the egg slowly elongates from this point, and the process terminates in the completion of the polar-bodies.<sup>1</sup> Preserved material shows that the first polar-body divides by mitosis. Vejdovský (19) has figured this in Taf. XIII, Fig. 8. Before, during, and after the formation

<sup>1</sup> One expression of a pathological condition of the egg is a constricting off at the periphery of small portions of its cytoplasm, and care is necessary to avoid confounding these with the polar-bodies.



of the polar-bodies, the egg turns slightly at indefinite intervals. These movements are not oscillatory, as sometimes three consecutive turns will occur in the same direction, revolving the egg one quarter its diameter. Later they appear to change the relative position of the egg and first polar-body, thus removing the first polar-body from the path of the second.

Pathological changes in the egg, which are induced sooner or later by the artificial examination medium, seem to appear first in the cytoplasm. An egg studied during the formation of the second polar-body (60 minutes) and for three hours thereafter, showed marked pathological changes in the cytoplasm. The polar rings did not develop, and the cytoplasm finally appeared as a mass of large vacuoles. During the period of these changes in the cytoplasm, the pronuclei continued to develop; for after killing, staining, and mounting the egg, the pronuclei were found to have reached their maximum size, and were in contact in the center of the egg, neither showing pathological features. The cytoplasm, however, showed the same pathological condition seen in the living egg.

As I am at work on a paper which will give the results of a comparative study of the living and fixed cytoplasm in these eggs, I shall omit here any description of the living, normal cytoplasm.

A large number of living eggs have been measured with a view to testing the shrinkage or swelling produced by various fixatives. The living oöcytes, 1st order, have varied in size from  $100\mu$  to  $132\mu$ , the oöcytes, 2d order, from  $114\mu$  to  $140\mu$ , the ripe egg from  $128\mu$  to  $144\mu$ , the pronuclear stages from  $136\mu$  to  $152\mu$ . These figures differ slightly from those given in my preliminary note (5), owing to the fact that the measurements were taken from fixed material. The eggs are not spherical at all stages; but these details will be illustrated later in a series of photomicrographs.

*Spermatozoa*.—Free spermatozoa can be procured by removing the slime-tube from copulating worms, drawing out part of its contents with a fine pipette, and drying on slides a thin layer of the seminal fluid thus obtained. They can be procured from a freshly deposited cocoon by drying on slides



a thin layer of the albumen of the cocoon. In this case, it is well to preserve and mount the eggs in the cocoon, in order to test the stage of development reached by the spermatozoa, by ascertaining whether the eggs are at the exact stage to be fertilized; for, as stated above, I have found freshly deposited cocoons containing eggs past the fertilization stage. Comparative measurements of the spermatozoa in the slime-tube, in the freshly deposited cocoon, and in the cocoon containing fertilized eggs, show only a slight difference in size. I wish here to correct a statement made in my preliminary note (5). I stated there that the full-grown spermatozoa taken from the freshly deposited cocoon were about  $2\frac{1}{2}$  times the length of those in the immature cocoon, and that they did not respond to differential staining. Further investigation has proved that the structures from which these measurements were taken are not normal spermatozoa, and as at the time I had not learned to control the investigation by preserving some of the eggs, I am convinced that these measurements were taken from spermatozoa in cocoons containing fertilized eggs. These spermatozoa of abnormal growth vary greatly in size, and are relatively far more numerous in the cocoons containing fertilized eggs. In some cases they appear to be developed by an abnormal growth of the head alone, while in others, though all parts select the same stain, the spine, head, and tail can be clearly identified. When stained with a chromatin and plasma stain, they, as a rule, select the latter; for example, with Biondi-Ehrlich all parts select the red, whereas the heads of the normal spermatozoa select the green. The large specimens suggest the giant spermatozoa of authors, though in this case they appear to be merely hypertrophied spermatozoa (text Fig. 1).<sup>1</sup> In the cocoons there are relatively few spermatozoa in which the spine, head, middle-piece, and tail respond to differential staining as do those in the slime-tube. Their

<sup>1</sup> In cocoons containing fertilized eggs, besides the individual spermatozoa, there appear to be attenuated masses of degenerating spermatozoa. Just such masses can be seen in certain spermathecae in which the spermatozoa show abnormal features, and in some cases from two to four heads are fused, making a relatively thick mass, whereas the tails are separated and can be counted, indicating just how many heads are fused.

number is very small as compared to the masses in the slime-tube and spermathecae, and for that reason I at first (erroneously) interpreted them as retarded individuals. I have since found that the head and tail of those in the fertilized egg respond to differential staining.

As many as nine spermatozoa have been found in one egg; but in such cases they do not all have an attraction-sphere. It



FIG. 1. — *A*, hypertrophied spermatozoön; *B*, normal spermatozoön.  
Camera.  $\times$  about 687.

appears as though the cytoplasm of the egg finally responded no longer to the stimulus. In many cases the tails can be identified within the fertilization cone. In two cases two spermatozoa are in the same cone without producing any apparent disturbance in its form.

#### SPERMATHECAE.

Authors agree in ascribing to the spermathecae the function of collecting the seminal fluid during copulation and storing it until it is needed for the cocoon, which has been supposed to

be formed by each worm subsequent to copulation.<sup>1</sup> The fact that both cocoons are formed *during* copulation seems to indicate that another function must be found for the spermathecae; and yet these, when crushed on a slide, stained and mounted, are found to be packed with spermatozoa. If one taken from a breeding worm is placed on a dry slide and pressed with a needle, its contents flow out in the form of a thick, viscid, cloudy fluid. Adding a few drops of water to this fluid and examining it under the microscope, shows it to be a mass of extremely active spermatozoa, the tails moving very rapidly and propelling the heads, which remain straight. When these spermatozoa are mounted and stained, comparative measurements indicate that they have reached the same stage of development as those in the spermatophores and seminal fluid of the slime-tube. Careful measurements have been taken of the various parts of several hundred spermatozoa found in the seminal vesicles, spermathecae, spermatophores, seminal fluid of the slime-tube and freshly deposited cocoon,<sup>2</sup> with the hope of determining whether the spermatozoa in the cocoon are from the spermathecae, but the differences in size were too slight to be of any value as a determining factor. An examination of the spermathecae of worms having just deposited their cocoons seemed to promise the only decisive answer. Thus far I have been able to examine the spermathecae of only three worms

<sup>1</sup> In studying the literature I have been able to find only one report that suggests the cocoons being formed during copulation, *viz.*, Vogt u. Yung. (Vergleichende Anatomie, pp. 481, 482). "Während der Begattung legen sich die beiden Würmer mit ihrer Bauchseite in entgegengesetzter Richtung derart an einander, dass der Kopf des einen dem Schwanz des anderen zugekehrt ist und dass die Geschlechtsöffnungen mit dem Gürtel wechselseitig in Berührung sind. Der in Form von kleinen weisslichen Massen ergossene Samen nimmt in zwei durch eine Vertiefung der Körperdecken gebildeten Längsrinnen die Gestalt kurzer Cylinder an und fliesst so zum Gürtel, um sich von dort in die Samentaschen zu begeben. Die beiden Würmer sind alsdann durch einen Ring von Schleim mit einander verbunden, der vom Gürtel und vielleicht auch von den Nebendrüsen abgesondert wurde, deren Gegenwart in der Nähe der Geschlechtsorgane wir erwähnt haben. Die durch die Mündungen der Eileiter ausgetretenen Eier gelangen zum Gürtel, wo sie von Schleim eingehüllt werden, in welchem man Samenthierchen wahrnimmt und der für sie eine Kapsel von eirunder Form bildet."

<sup>2</sup> In these the length of the spine has been found to vary from  $4\mu$  to  $7\mu$ , head  $24\mu$  to  $34\mu$ , middle-piece  $2\mu$  to  $5\mu$ , tail  $54\mu$  to  $67\mu$ . The longest head found in the fertilized egg measured  $34\mu$ .

with nearly completed cocoons, and in all these cases the sacs were found to be empty and flattened. In some cases a large mass of spermatophores was aggregated at one end of the slime-tube, covering segments 9 to 11 of one worm, while the same segments of the other worm showed no spermatophores. In the former case the spermathecal sacs were empty; in the latter, full. An examination of the spermathecae of twenty worms that were not copulating showed only one with empty spermathecae. This indicates that the sacs are not filled and emptied during one copulation. The above facts seem rather to necessitate two copulations, one to fill the spermathecae, and one to form the cocoons. I have several times watched copulating worms from three to five hours consecutively, and seen them separate without being able to identify a cocoon in any stage of formation. Copulating worms are, however, so

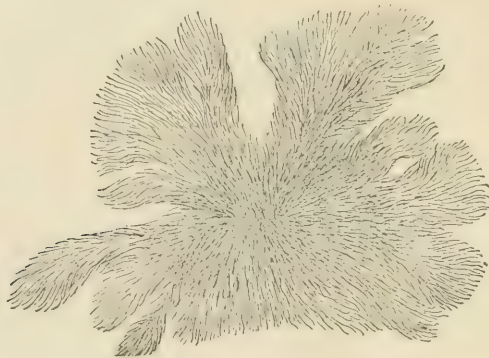


FIG. 2. — Spermatophore.  $\times 150$ .

easily disturbed that it would be necessary to repeat this observation a great many times, as well as to examine the spermathecae after the worms have separated, before accepting such observation in proof of a second copulation.

#### SPERMATOPHORES (text Fig. 2).

The spermatophores are formed after the spermatozoa leave the spermathecae. The contents of the latter when stirred with a needle in water will disintegrate, each spermatozoön



becoming individualized and the mass forming an approximately homogeneous fluid, whereas the spermatophores do not disintegrate in water, even after twenty-four hours' immersion. This suggests that the spermatozoa, after leaving the spermathecae, come in contact with some adhesive substance which welds them into masses and confines them within the area which is occupied later by the cocoon. Removing the slime-tube from

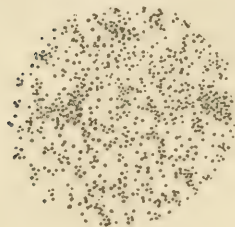


FIG. 3.  $\times 500$ .

worms with partly formed cocoons, I found on the surface of segments 9 to 11, inclusive, tiny opaquely white specks (sometimes as many as nine) apparently issuing from integumental orifices. These, like the spermatophores, did not disintegrate in water, and when stained and mounted they showed a very definite granular structure (text Fig. 3).

The glands which secrete this substance can be seen on dissecting the worm from the ventral surface and removing everything but the spermathecae, leaving the inner surface of the integument exposed. On segments 9 to 11, inclusive, are tiny opaquely white swellings. If these are pricked with a needle, an opaquely white substance can be pressed from them, which, when stained and mounted, entirely resembles that described above as found on the exterior of the same segments. In some worms these tiny swellings are quite numerous, one being close to the stem of each spermatheca and at least four in each of the three segments, these being distributed in the center of the segment as well as close to the dissepiments.

If the center, more dense part, of a spermatophore is crushed on a slide, stained and mounted, we find the same granular secretion as in the above-mentioned integumental glands of segments 9 to 11, and on their exterior surface. Thus it appears to be demonstrated that the spermatophores of this worm are formed by the spermatozoa aggregating around the granular—probably nutritive—substance secreted by these glands.

*Archoplasm.*—The appearance of the archoplasm (Foot (6)) in the living egg indicates that it is at least semi-fluid, and this interpretation is in accord with the history of the archoplasm,



as traced in fixed material. Its relatively rapid change of position in the egg, its accumulation at all the centers of activity, — spindle, cone, and sphere, — its subsequent aggregation at the periphery, and its final massing at the poles indicate that it is not a mere condensation of the cytoplasmic network; for the migration of such points of condensation from the periphery to the poles would cause a marked disturbance of the network. The differentiation of a portion of the archoplasm in both spindle and spheres supports the observations of those investigators who have differentiated a specific substance in either of these two structures. Strasburger (18), Boveri (2), Meves (13–14), George Niessing (16), Carl Niessing (15), Henneguy (10), and von Klinckowström (12). Whether the archoplasm can be identified with the “hyalinen Grundsubstanz” Vejdovský (19), p. 40, has observed in these eggs, I am unable to determine. Thus far I have been unsuccessful in attempts to differentiate anything else that suggests the substance in question; but this fact can scarcely be regarded as evidence of its identity with archoplasm; neither have attempts to differentiate the cell-sap of authors proved successful; but I am not at all prepared to say that for this reason the archoplasm must be identified with the cell-sap.

The fate of that portion of the archoplasm which forms the polar rings has not yet been ascertained; neither am I prepared at present to discuss the difference in form between the two rings, nor the lack of constancy in the form of either ring. At the pronuclear stage, — when the two masses are complete, — their position in relation to each other appears to be constant. The cleavage-planes, however, stand in no constant relation to these structures (the substance is not divided between the cells), and even the first cleavage sometimes assigns both polar rings to one cell, no part of them being consigned to the other.

*Centrosome.* — Since expressing the belief that the cleavage centrosomes are not derived from the middle-piece of the spermatozoön (7), and that the middle-piece (the posterior end of the head of the spermatozoön) produces an effect upon the cytoplasm comparable to the effect produced on it by the

spine (the anterior end of the head of the spermatozoön), I have made the following observations on this point. I have seen very early stages of the development of the sperm attraction-sphere, — stages where the middle-piece of the sperm is still intact, while the rays of the attraction-sphere are focused around it, just as the rays of the cone are at one stage focused around the head of the spermatozoön. I hope to be able to illustrate the morphological resemblance of these two structures in a series of photomicrographs.

*Cytoplasmic Granules or Microsomes.*—In an earlier paper (7) I published a list of structures in these eggs, which select methyl green, among them numerous “large and small granules or bodies,” also the centrosomes and nucleoli. I have since succeeded in differentiating from these structures the centrosomes and the nucleoli, the latter being found in the cytoplasm of the oöcytes, 1st and 2d orders, as well as in the pronuclei. The details of this work will be published later.

*Spindle.*—The phenomena in this egg indicate that at least part of the first cleavage spindle has its origin in the cytoplasm; as the polar rays are often formed while the membrane of both pronuclei is intact. When an isolated male pronucleus has reached its maximum size, its attraction-sphere is seen in the cytoplasm, while the membrane of the pronucleus is still intact. Occasionally a spindle is formed by the meeting of the rays of two male attraction-spheres, or the rays of a male attraction-sphere with those of the egg attraction-sphere at the lower pole of the second maturation spindle. All these rays anastomose, as do the fibers in the first and second maturation spindle.<sup>1</sup>

*Reduction.*—Twenty-two chromosomes can be counted in the oögonia and only eleven in the first maturation spindle; thus we have the typical number reduction of chromosomes, — the “pseudo-reduction” of Rückert (17). The position and shape of the chromosomes in the spindle accord with Flemming’s (4) heterotype form of division. In many cases four distinct parts can be differentiated (text Fig. 4), the typical tetrad being thus represented. At the beginning of the anaphase

<sup>1</sup> For figures of these spindles, see Foot (5), Figs. 1 and 3; (8), Fig. 4.

of the second spindle, each of the eleven chromosomes is represented by two bent, straight, or contracted rods,<sup>1</sup> this longitudinal division resembling that of the twenty-two chromosomes of the cleavage spindle. The phenomena in this egg offer no evidence in support of Häcker's (9) last theory of reduction; for to meet the demands of this theory there must be some evidence that the two parts of each chromosome of the second spindle are individually the same two parts of the tetrad that remained in the egg after the first division. This cannot be asserted, for the reason that between the first and second divisions we have a semi-resting stage,<sup>2</sup> that is, the chromosomes take the first steps towards a typical resting stage, forming vesicles *entirely similar* to those formed after the second division and after the first cleavage. There are eleven vesicles. This indicates that two parts of each tetrad of the first spindle have fused into one vesicle, and the individuality of each is thus lost. The process of the reorganization of these vesicles into the chromosomes of the second spindle must occur very rapidly; for none of my preparations show this step in the development.

This egg appears to show only two methods of reduction, — first, the above-mentioned number reduction, and second, a mass reduction. The latter is proved by the omission of a typical resting stage and by the amount of chromatin in the second spindle as compared with that in the first. In text Fig. 4 are represented some of the forms shown by the tetrads of the first maturation spindle. I am unable at present to give any data regarding the formation of these tetrads, as I have not

<sup>1</sup> For figures representing these chromosomes, see Foot (5), Fig. 3; (6), Fig. 10; (8), Fig. 4.

For polar view of the tetrads (5), Fig. 4, and Fig. 5 for polar view of second maturation spindle. At the time these last two figures were drawn, I was unable to interpret the apparent tetrads often found in the second maturation spindle. The following summer, in preparations differently fixed, the rods showed a succession of bead-like formations (6 or more), this indicating that the swelling of each rod into two or several of these bead-like formations is possibly due to the fixative or some other artificial factor.

<sup>2</sup> I designate this as a semi-resting stage, for the reason that the chromatin lost by the last division is not replaced by growth, this being an essential feature of the typical resting stage.

yet attempted to collect eggs showing these stages. Undoubtedly, however, they can be found at some stage during copulation, either in the worm, or in the slime-tube. It is necessary to obtain these stages before deciding whether the first divi-

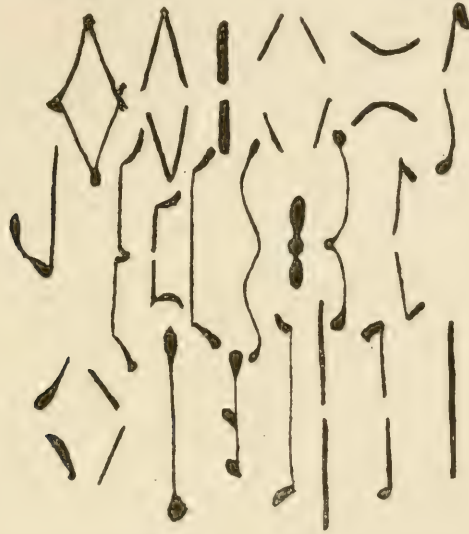


FIG. 4. — Some forms shown by the chromosomes of the first maturation spindle.

sion is longitudinal or transverse, though the form of the tetrad suggests that the first division is a longitudinal splitting of two chromosomes attached end to end.

I am in hopes that further work upon this material may enable me to obtain more definite results in several directions where at present I feel dissatisfied.

I take pleasure in expressing my great obligation to Dr. Whitman for critical examination of my manuscript and for many other courtesies in connection with this paper.



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## EXPLANATION OF PLATE XXXVIII.

FIG. 1. Anterior segments of two copulating worms, slime-tube encircling the pair from the 8th to the 33d segments.  $\times 3\frac{1}{2}$ . Seminal fluid between the two worms coagulated by the reagents. At the distal ends of the tube an aggregation of spermatophores.

FIG. 2. Slime-tube containing seminal fluid and spermatophores. Preserved after the copulating worms have been disturbed and allowed to withdraw.  $\times 3\frac{1}{2}$ .

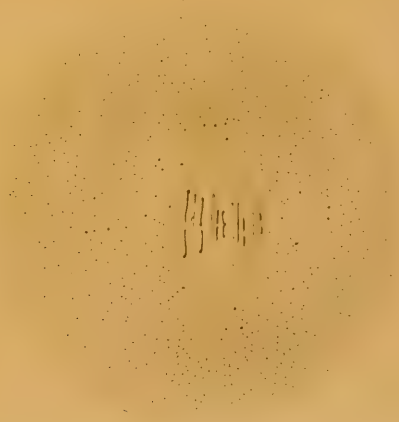
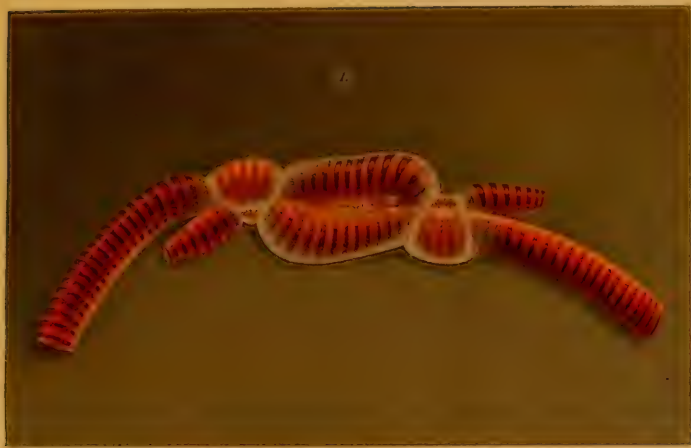
FIG. 3. Freshly deposited cocoon, surrounded by one-half of a slime-tube.  $\times 7$ .

FIG. 4. Older cocoon, probably containing eggs in the pronuclear stages.

FIG. 5. Section of an unfertilized egg, from a freshly deposited cocoon.  $\times$  about 687.























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